The
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Founded by HENRY BALDWIN WARD

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## Program and Abstracts of the 30th Annual Meeting, American Society of Parasitologists, Atlanta, Georgia, December 27, 28, 29, 30, 1955.

Copies of this publication will be available at Atlanta

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## THE JOURNAL OF PARASITOLOGY

The Journal of Parasitology, published bi-monthly, is the official organ of the American Society of Parasitologists and as such it publishes the program of the annual scientific meeting, abstracts of the papers presented there, and minutes of the business meetings of the Society.

The Journal of Parasitology is a medium for the publication of the results of research in the field of animal parasitology. Manuscripts dealing with subjects in this field will be considered for publication, and accepted manuscripts will be published in order of receipt. A manuscript is accepted with the understanding that it is to be published exclusively in the Journal unless otherwise arranged in advance.

Articles submitted for publication may be sent to any member of the Editorial Committee or Editorial Board. Responsibility for acceptance of papers is divided in the Editorial Committee for the fields of helminthology, protozoology and entomology. Correspondence concerning the disposition or revision of manuscripts should be addressed to George R. La Rue, Chairman of the Editorial Committee, Parasite Research Laboratories, A.R.S., Beltsville, Maryland.

Manuscripts submitted should be typewritten, double or triple spaced, with wide margins, and with tables, graphs, and illustrations in finished form for transmission to the printer. Title, author, institution, etc., go on a separate sheet. Each table requires a separate sheet. Footnotes are not encouraged. Such material can usually be embodied in the text. If footnotes are used, they should be numbered consecutively and typed on a sheet separate from the text. Authors are urged to emphasize simplicity and clearness in tables. When feasible, sharp, contrasty photographic reproductions of large plates and graphs should be submitted in place of the originals. In preparing plates care should be given to the effective arrangement of figures, to economy of space, and to the amount of reduction required. Figures should be arranged within an area five by eight inches, or multiples thereof. Legends for figures should be typed on a sheet, separate from the text, and containing no illustrations. Greek characters and symbols should be avoided, where possible, since they require hand composition, thus increasing the cost of publication.

The bibliography, double or triple spaced, should contain only those references actually cited in the text, and its style should conform to bibliographies in recent issues of this Journal. The Index Catalogue of Medical and Veterinary Zoology should be used as a guide for abbreviating the journals cited.

Since it is the desire of the Editorial Committee to publish all acceptable manuscripts submitted within the limited space available, authors are urged to present their results briefly, avoiding tables where possible. However, acceptability of a paper will be determined not by its length but by the amount and character of new information included and the form in which it is presented. Long articles, and those with an unusual number of tables and plates, can be published only with the financial cooperation of the author.

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# The Journal of Parasitology

Vol. 41, No. 6, Section 2 (Supplement) December, 1955


## PROGRAM AND ABSTRACTS OF THE THIRTIETH ANNUAL MEETING OF THE AMERICAN SOCIEY OF PARASITOLOGISTS

ATLANTA, GEORGIA

December 27, 28, 29, 30, 1955

NOTE: Registration: 9:00 A.M. to 5:00 P.M. Tuesday through Friday—Atlanta Municipal Auditorium. Get Luncheon and Bus Tickets here.

### *PROGRAM*[1]

TUESDAY EVENING, DECEMBER 27, 6:30 P.M., PANEL C, DINKLER PLAZA HOTEL. COUNCIL DINNER AND MEETING, OFFICERS AND MEMBERS OF THE SOCIETY'S COUNCIL.

(All Past Presidents of the Society are cordially invited to attend both the dinner and the meeting.)

WEDNESDAY MORNING SESSION, DECEMBER 28, 9:00 A.M., MIRROR ROOM, DINKLER PLAZA, ARTHUR C. WALTON, KNOX COLLEGE, PRESIDING.

Presentation of papers:

*Read*

(Authors are requested to keep within the time limits indicated for each paper)

1. An intestinal parasite survey of the Korean Prisoner of War Camp located on Koje Island. (10 min.) (2 × 2 projector). M. M. BROOKE, Communicable Disease Center; J. C. SWARTZWELDER, Louisiana State University; F. J. PAYNE, CDC; P. WEINSTEIN, NIH; AND W. W. FRYE, Louisiana State University.

2. Treatment of asymptomatic amebiasis with atabrine in combination with carbarsone and with chiniofon. (10 min.) (Standard projector). T. SASKI, Yamanashi Medical Research Institute, Kofu, Japan; D. E. WYKOFF, Army Medical Service Graduate School; M. YOKOGAWA, Institute of Public Health, Tokyo, Japan; and L. S. RITCHIE, 406th Medical General Laboratory, APO 500, San Francisco, California.

3. Cultivation of *Entamoeba histolytica* from trophozoites in fecal suspensions kept at 25° C. for 4 days. (10 min.). M. GOLDMAN AND R. K. CARVER, CDC.

[1] An alphabetical author-index will be found at the end of this section of the program. Extra copies of this supplement will be on sale at the meeting.

4. Measuring the brightness of individual amebae exposed to fluorescein-tagged antibodies. (10 min.) (Standard projector). M. GOLDMAN, CDC.

5. Virulence studies with a strain of *Entamoeba histolytica* maintained *in vitro* in liquid media. (10 min.) (Standard projector). D. J. TAYLOR AND J. GREENBERG, NIH.

6. Blood-induced blackhead. (10 min.). W. C. MCGUIRE, Dr. Salsbury's Laboratories.

7. A summer survey of the blood parasites of two *Microtus pennsylvanicus* populations, Ithaca, N. Y. (10 min.). S. H. KIRNER, K. R. BARBEHENN AND B. V. TRAVIS, Cornell University.

8. Incidence studies on *Trypanosoma diemyctyli* Tobey, 1906, in *Diemyctylus v. viridescens* (Raf.). (7 min.) (2×2 projector). J. S. MACKIEWICZ, Cornell University.

9. Biochemical studies of chicken macrophages infected with *Trypanosoma cruzi* (Chagas). (10 min.) (Standard projector). L. G. WARREN, Johns Hopkins University.

10. Growth factor in normal rat serum for *Trypanosoma lewisi.* (10 min.) (Standard projector). D. R. LINCICOME, Naval Medical Research Institute.

11. Observations on the immunity to *Trypanosoma equiperdum* induced by treatment with Oxophenarsine. (10 min.) (Standard projector). W. CANTRELL AND G. D. BETTS, University of Louisville.

12. Comparative studies on the physiology of trichomonad flagellates. (10 min.) (Standard projector). C. P. READ, Johns Hopkins University.

13. The effect of cysteine on growth of *Trichomonas vaginalis.* (8 min.) (Standard projector). B. F. SMITH, Spelman College.

WEDNESDAY AFTERNOON SESSIONS, DECEMBER 28, 2:00 P.M.

SECTION 1. *PROTOZOA*, MIRROR ROOM, DINKLER PLAZA, E. E. BYRD, UNIVERSITY OF GEORGIA, PRESIDING.

*Read*

(Please abide by the time limits)

14. Experimental treatment of bovine trichomoniasis in bulls with acriflavine and other agents. (10 min.) (Standard projector). P. A. FITZGERALD, USDA; and J. THORNE, A. E. JOHNSON AND D. M. HAMMOND, Utah State Agricultural College.

15. Experimental infections of the bovine genital tract with trichomonads from swine. (10 min.) (Standard projector). P. A. FITZGERALD, USDA; AND A. E. JOHNSON, J. THORNE AND D. M. HAMMOND, Utah State Agricultural College.

16. Further observations on occurrence of *Hepatozoon* in gray squirrels (*Sciurus carolinensis*). (10 min.) (Standard projector). C. M. HERMAN, Patuxent Research Refuge; AND G. M. CLARK, University of Maryland.

17. The development of *Hepatozoon sciuris* Coles, 1914, in two parasitic laelaptid mites. (10 min.) (2×2 projector). G. M. CLARK, University of Maryland. (Introduced by G. W. WHARTON, University of Maryland).

18. Sulfaquinoxaline and sulfamerazine in the treatment of experimental infections with *Eimeria bovis* in calves. (10 min.) (Standard projector). D. M. HAMMOND, J. L. SHUPE, A. E. JOHNSON AND J. THORNE, Utah State Agricultural College; and P. A. FITZGERALD, USDA.

19. Size of the oocyst of *Eimeria brunetti* and *E. necatrix*. (10 min.) (Standard projector). E. R. BECKER, W. H. PATTILLO, J. R. FARMER AND W. M. VAN DOORNINCK, Iowa State College.

20. The effect of cecal coccidiosis on the motility of the digestive tract of the domestic fowl. (10 min.) (Standard projector). C. S. SCHILDT, Henderson State Teachers College; AND C. A. HERRICK, University of Wisconsin.

21. A comparative study of the effects of cecal coccidia on the ability of plymouth rock and leghorn chickens to do muscular work. (10 min.) (Standard projector). L. LEVINE, Wayne University; AND C. A. HERRICK, University of Wisconsin.

22. The effect of nicarbazin on avian COCCIDIA. (10 min.) (Standard projector). A. C. CUCKLER AND C. M. MALANGA, Merck Institute for Therapeutic Research.

23. Comparative susceptibility of erythrocytes from mammals and birds to infections by four species of avian malaria. (10 min.) (Standard projector). R. B. MCGHEE, University of Georgia.

24. Further observations on the transmission and development of *Leucocytozoon simondi* M & L. (10 min.) (2×2 projector). A. M. FALLIS, R. C. ANDERSON AND G. F. BENNETT, Ontario Research Foundation.

25. Relationship of size of inoculum to time to death in mice infected with *Toxoplasma gondii*. (10 min.) (Standard projector). D. E. EYLES AND N. COLEMAN, NIH, Memphis, Tenn.

26. Immunity in murine toxoplasmosis. (10 min.) (Standard projector). L. JACOBS AND M. L. MELTON, NIH, Bethesda, Md.

27. Parasitological and serological studies on the prevalence of canine toxoplasmosis in Memphis, Tennessee. (10 min.) (Standard projector). C. L. GIBSON AND J. R. JUMPER, NIH, Memphis, Tenn.

28. The therapeutic value of fumagillin in the control of *Nosema apis* Zander in the honey bee. (10 min.) (Standard projector). H. E. CMEJLA, Abbott Laboratories; C. A. HERRICK, University of Wisconsin; AND C. L. FARRAR, University of Wisconsin and the USDA.

*Read by Title Only*

29. Cytochemical observations on cultured macrophages infected with *Leishmania donovani*. C. P. READ AND P. CHANG, Johns Hopkins University.

30. Repeated enhancement in virulence of a strain of *Toxoplasma* by passage in mice. M. L. MELTON AND L. JACOBS, NIH, Bethesda, Md.

SECTION 2. *TREMATODA*, PANEL ROOMS C AND D, DINKLER PLAZA, D. V. MOORE, SOUTHWESTERN MEDICAL SCHOOL, PRESIDING.

*Read*

(Please abide by the time limits)

31. Life-cycle of *Diplostomum* (*baeri?*) (Trematoda: Strigeida). (10 min.) (Standard projector). G. L. HOFFMAN, University of North Dakota.

32. Studies on the life-cycle and development of *Crassiphiala bulboglossa* (Trematoda: Strigeida.) (10 min.) (Standard projector). G. L. HOFFMAN, University of North Dakota.

33. Growth and development of *Schistosoma mansoni* in mice. (10 min.) (Standard projector). M. G. RADKE, M. D. SCHNEIDER AND D. G. HOUGHTALING, SU 3004, Third Army Area Medical Laboratory, Fort McPherson, Georgia.

34. The structure and source of the pericercarial envelope (CHR) of *Schistosoma mansoni*. (10 min.) (Standard and 2×2 projectors). F. J. KRUIDENIER, University of Illinois; AND M. A. STIREWALT, Naval Medical Research Institute.

35. Penetration of host skin by cercariae of *Schistosoma mansoni*. I. Observed entry into skin of mouse, hamster, rat, monkey and man. (10 min.) (Standard projector). M. A. STIREWALT, Naval Medical Research Institute.

36. Serologic reactions in *Schistosoma mansoni* infections: ionographic fractionation of sera of mice with progressive disease. (10 min). (Standard projector). A. S. EVANS AND M. A. STIREWALT, Naval Medical Research Institute.

37. Intradermal reactions to *Schistosoma japonicum* and *S. mansoni* antigens in schistosome dermatitis cases. (10 min.). H. F. HSÜ, State University of Iowa; AND D. J. AMEEL, Kansas State College.

38. Immunologically reactive substance from *Schistosoma mansoni*. (10 min.) (Standard projector). M. D. SCHNEIDER, M. G. RADKE AND M. T. COLEMAN, SU 3004, Third Army Area Medical Laboratory, Fort McPherson, Georgia.

39. Studies on schistosomiasis. XII. Results of experiments on screening potential protective ointments on mice against the cercariae of *Schistosoma mansoni*. (10 min.) (Standard projector). G. W. HUNTER, III, Fourth Army Area Medical Laboratory, Fort Sam Houston, Texas; AND H. A. KEMP, H. E. SMALLEY, O. P. WILKINS AND C. DIXON, Baylor University.

40. Chromosomes and sex determination in *Schistosomatium douthitti* (Trematoda: Schistosomatidae). (10 min.) (Standard projector). R. B. SHORT, Florida State University.

41. Chromosomes in parthenogenetic miracidia of *Schistosomatium douthitti* (Trematoda: Schistosomatidae) and their progeny in snails. (10 min.) (Standard projector). R. B. SHORT AND M. Y. MENZEL, Florida State University.

42. Studies on immunity to *Fasciola hepatica* in rabbits. (10 min.). G. R. HEALY, Rice Institue.

43. Affinities of the trematode family DIDYMOZOIDAE. (10 min.). R. M. CABLE, Purdue University.

44. Studies on the germ cycle of an ochetosomatid trematode. I. Gametogenesis in *Neorenifer*. (10 min.) (Standard projector). M. C. DUNN, University of Georgia.

*Read by Title Only*

45. Preliminary studies on haemagglutination in schistosomiasis. I. G. KAGAN, University of Pennsylvania; AND J. OLIVER-GONZÁLEZ, University of Puerto Rico.

46. Use of organ egg counts in assaying chemotherapeutic activity against *Schistosoma mansoni*. T. W. SCHWINK, Tropical Research Medical Laboratory, APO 851, New York, N. Y.

47. Field-test of four molluscacides against *Oncomelania nosophora* in the terraced-hillside habitat. A. P. MOON, Y. OTORI, J. E. WILLIAMS, L. P. FRICK AND L. S. RITCHIE, 406th Medical General Laboratory, APO 500, San Francisco, Calif.

48. Applications of molluscacides against juvenile *Oncomelania nosophora* for

repopulation control. J. E. WILLIAMS, A. P. MOON, Y. OTORI, L. P. FRICK AND L. S. RITCHIE, 406th Medical General Laboratory, APO 500, San Francisco, Calif.

49. A restudy of the genus *Allassogonoporus* (Oliver, 1938) Macy, 1940. J. H. GILFORD, Johns Hopkins University.

50. Studies to maintain excysted metacercariae of *Paragonimus westermani in vitro.* M. YOKOGAWA, T. OSHIMA AND M. KIHATA, Institute of Public Health, Japan.

51. Respiration of *Paragonimus westermani.* C. P. READ AND M. YOGORE, Johns Hopkins University.

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NOTE: The following events occurring Wednesday may be of general interest to our members:

2:00 P.M.—Symposium, Committee Room 2, Municipal Auditorium.
"The Species Concept."

8:00 P.M.—AAAS Presidential Address, Auditorium, Altanta University.
Warren Weaver, President, AAAS.

9:00 P.M.—AAAS Reception, Gymnasium, Altanta University.
Presidential Reception.

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THURSDAY MORNING SESSIONS, DECEMBER 29, 9:00 A.M.

SECTION 1. *CESTODA,* MIRROR ROOM, DINKLER PLAZA, ALLEN MCINTOSH, USDA, PRESIDING.

*Read*

(Please abide by the time limits)

52. Notes on the anatomy of *Ligula intestinalis* (Linnaeus, 1758), a pseudophyllidean cestode of birds. (10 min.) (Standard projector). E. E. BYRD, University of Georgia.

53. Studies on *Echinococcus sibiricensis* (Rausch and Schiller, 1954) in laboratory mice. (12 min.) (2×2 projector). S. K. MANKAU, University of Illinois.

54. Echinococcosis on St. Lawrence Island, Alaska. (12 min.) (2×2 projector). L. J. THOMAS, University of Illinois and the USAF Arctic Aeromedical Laboratory.

55. Immunity to *Hydatigera taeniaeformis* affected by radiation of the host. (10 min.) (2×2 projector). H. CIORDIA AND A. W. JONES, University of Tennessee.

56. The *in vitro* fermentation of carbohydrate substrates by *Hymenolepis diminuta* and the action of some inhibitors. (12 min.) (Standard projector). J. S. LAURIE, Johns Hopkins University.

57. Studies on lipid metabolism in cestodes. (7 min.) (Standard projector). M. WARREN AND J. W. DAUGHERTY, Rice Institute.

58. The effect of fasting and Alloxan treatment of the host on glyogen gradient in *H. diminuta.* (8 min.) (Standard projector). J. W. DAUGHERTY, Rice Institute.

59. A comparison of the amino acid constituents and the transaminase activity of *Raillietina cesticillus* and *Hymenolepis diminuta.* (8 min.) (Standard and 2×2 projectors). W. B. FOSTER, Rice Institute.

*Read by Title Only*

60. The relation between lesions in lambs and the development of the cysticerci

of *Taenia hydatigena* Pallas, 1766. G. K. SWEATMAN, Canada Department of Agriculture.

61. Glycogen synthesis in *Hymenolepis diminuta.* J. W. DAUGHERTY, Rice Institute.

62. Experimental demonstration of the life cycle of *Hymenolepis citelli* (McLeod, 1933) (Cestoda: Cyclophyllidea). M. VOGE, UCLA.

63. Studies in embryology and histology of the robin tapeworm, *Choanotaenia iola* Lincicome (Cyclophyllidea: Dipylidiinae), with description of the metachromatic glands in the onchosphere. R. E. OGREN, Ursinus College.

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SECTION 2. *ANNELIDA and ARTHROPODA,* PANEL ROOMS C AND D, DINKLER PLAZA, GILBERT F. OTTO, PRESIDING.

*Read*

(Please abide by the time limits)

64. The "nasal-leech" *Dinobdella ferox* from Borneo and Malaya. (10 min.) (Standard projector). B. C. WALTON, Army Medical Service Graduate School.

65. Factors influencing the susceptibility of mosquitoes to malarial parasites. (10 min.) (Standard projector). D. W. MICKS, University of Texas Medical Branch at Galveston.

66. Quantitative microinjection of mosquitoes. (10 min.) (Standard projector). J. CHAO AND G. H. BALL, UCLA.

67. Sterile microdissection and isolation of malarial oocysts from the mosquito stomach. (10 min.) (Standard projector). J. CHAO, UCLA.

68. The tergal plates of North American *Anopheles* larvae—their use in identification and phylogenetic studies (Diptera: Culicidae). (10 min.) (Standard projector). H. D. PRATT, CDC, Atlanta.

69. Studies on the clinical effects of the screw-worm, *Callitroga hominivorax* (Coquerel) on guinea pigs. (10 min.) (Standard projector). J. H. ESSLINGER, Rice Institute.

70. Experimental studies on argasid ticks as possible vectors of tularemia. (10 min.) (2×2 projector). W. BURGDORFER, Rocky Mountain Laboratory. (Introduced by C. B. Philip, Rocky Mountain Laboratory).

71. Food preference and longevity of the spiny rat mite, *Echinolaelaps echidninus.* (10 min.) (2×2 projector). R. W. STRANDTMANN AND VIRGINIA LANDWER, Texas Technological College.

*Read by Title Only*

72. Observations on the life-cycle of the parasitic mite *Chorioptes bovis* (Gerlach) using an *in vitro* technique. G. K. SWEATMAN, Canada Department of Agriculture.

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THURSDAY MORNING SESSION, DECEMBER 29, 11:15 A.M.

*Presidential Address,* RAINBOW ROOF, DINKLER PLAZA HOTEL.

73. "Parasitism and Parasitology" (40 min.). CLAY G. HUFF, Naval Medical Research Institute, Bethesda, Maryland, President of the American Society of Parasitologists. Introduction by GILBERT F. OTTO, Abbott Laboratories, North Chicago, Illinois, Vice-President of the Society.

THURSDAY NOON, DECEMBER 29, 12:10 P.M., RAINBOW ROOF, DINKLER PLAZA. ANNUAL LUNCHEON AND BUSINESS MEETING OF THE SOCIETY.

C. G. HUFF, PRESIDING.

(get tickets at time of registration)

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THURSDAY AFTERNOON, DECEMBER 29, 2:30 P.M. Bus leaves DINKLER PLAZA for trip to COMMUNICABLE DISEASE CENTER, CHAMBLEE, GEORGIA. M. M. BROOKE in charge. Registration must be in advance—bus will return direct to EMORY UNIVERSITY.

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THURSDAY AFTERNOON SESSION, DECEMBER 29, 3:15 P.M. BIOLOGY LABORATORIES, EMORY UNIVERSITY. C. G. GOODCHILD IN CHARGE. (Chartered buses will leave the hotel at 3:15 and return from the University at 5:15. Purchase tickets when you register)

*All Papers by Demonstration and Exhibit*

74. Development of megaloschizonts of *Leucocytozoon simondi*, Mathis & Léger, 1910, and some host reactions. A. B. COWAN, Patuxent Research Refuge. (Introduced by C. M. HERMAN, Patuxent Research Refuge).

75. Precipitates on the larvae of *Litomosoides carinii* in sera of repeatedly infected white rats. N. T. BRIGGS, University of Texas Medical Branch at Galveston.

76. Some microfilariae from birds, with a graphic demonstration of species differences. E. J. ROBINSON, JR., Kenyon College.

77. Anomalies in *Fasciola hepatica*. G. R. HEALY, Rice Institute.

78. The life-cycle of *Azygia sebago* Ward, 1910. H. W. STUNKARD, New York University.

79. The gland complex of the cercaria of *Schistosoma mansoni*. F. J. KRUIDENIER, University of Illinois; AND M. A. STIREWALT, Naval Medical Research Institute.

80. The nervous system of some DIGENEA. N. KINGSTON AND D. L. DEGIUSTI, Wayne University.

81. Morphology of the adult and the miracidium of a progenetic species of *Allocreadium* from water beetles of the family DYTISCIDAE. L. E. PETERS, JR., Purdue University.

82. Encephalitis in a swan due to *Dendritobilharzia* sp. N. D. LEVINE, D. T. CLARK AND L. E. HANSON, University of Illinois.

83. *Nematobothrium texomensis* n. sp. from a freshwater fish, *Ictiobus bubalus* (Rafinesque, 1819). A. MCINTOSH, USDA; AND J. T. SELF, University of Oklahoma.

84. Transfaunation of adult *Hydatigera taeniaeformis* to its intermediate host. A. W. JONES AND H. CIORDIA, University of Tennessee.

85. Acanthocephala of the genus *Centrorhynchus* from birds of Egypt. H. L. WARD, University of Tennessee.

86. Nymphal stages of *Pneumonyssus simicola* (Acarina: Halarachnidae). W. B. HULL, Naval Medical Research Institute. (Introduced by A. C. WALTON, Knox College).

87. Penetration of host tissue and host reaction to chigger mites of the genus *Hannemania* (Acarina: Trombiculidae). K. E. HYLAND, JR., University of Rhode Island; AND G. W. WHARTON, University of Maryland.

88. An apparatus for the continuous collection of ectoparasites from nests of small mammals. R. O. DRUMMOND, University of Maryland.

*The following films will be shown during the Demonstration Session.*

89. "Schistosomiasis in Egypt." R. E. KUNTZ, Naval Medical Research Institute.

90. A motor-driven enterotome for the rapid removal of helminths from the gastrointestinal tracts of large mammals. R. F. SHUMARD, I. A. SCHIPPER AND D. F. EVELETH, North Dakota Agricultural Experiment Station.

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NOTE: The following events occurring Thursday may be of general interest to our members:
9:00 A.M.—AAAS General Session, Plaza Room, Dinkler Plaza.
"Shortage of Scientists."
2:00 P.M.—Symposium, Committee Room 2, Municipal Auditorium.
"The Species Concept."
6:30 P.M.—Zoologists' Dinner, Atlanta University. Followed by address by the Vice-President of Section F, AAAS.—J. Gordon Carlson.
8:30 P.M.—Biologists' "Smoker," Municipal Auditorium.

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FRIDAY MORNING SESSION, DECEMBER 30, 9:00 A.M., MIRROR ROOM, DINKLER PLAZA, R. M. CABLE, PURDUE UNIVERSITY, PRESIDING.

*Read*

(Please abide by the time limits)

91. Studies on the susceptibility of hamsters to *Trichinella spiralis*. (10 min.) (2×2 projector). E. H. SADUN AND L. NORMAN, CDC, Chamblee.

92. Establishment of *Trichinella spiralis* infections in Thiry-Vella fistularized rats. (12 min.). C. G. GOODCHILD AND W. BOODISH, Emory University.

93. Further observations on phthalofyne as a trichuricide. (10 min.) (Standard projector). F. A. EHRENFORD, G. BURCH AND C. BUNDE, Pitman-Moore, Co.

94. The storage and standardization of sera and reagents for the diagnosis of trichinosis by the bentonite flocculation test. (10 min.) (2×2 projector). L. NORMAN, CDC, Chamblee. (Introduced by M. M. BROOKE, CDC, Chamblee).

95. Determinations of the phosphorus content of golden nematode and root-knot nematode. (12 min.) (Standard projector). V. H. DROPKIN, U. S. Nematode Research Laboratory, USDA, Seaford, N. Y.

96. The life history of *Toxascaris leonina* in the cat. (5 min.) (2×2 projector). J. F. A. SPRENT, University of Queensland. (Read by P. C. BEAVER, Tulane).

97. The life history of *Toxacara cati* in the cat. (5 min.) (2×2 projector). J. F. A. SPRENT, University of Queensland. (Read by P. C. BEAVER, Tulane).

98. The life history of *Ophidascaris filaria* in the carpet snake (*Morelia argus*). (5 min.) (2×2 projector). J. F. A. SPRENT, University of Queensland. (Read by P. C. BEAVER, Tulane).

99. The effectiveness of piperazine citrate against intestinal parasites, especially *Ascaris*, in mental patients. (10 min.) (Standard projector). M. D. YOUNG, NIH, Columbia, S. C.; AND J. E. FREED, South Carolina State Hospital.

100. Piperazine citrate as an anthelmintic in domestic animals. (12 min.)

(2×2 projector). R. F. SHUMARD AND D. F. EVELETH, North Dakota Agricultural Experiment Station.

101. The value of repeated examinations in the diagnosis of infections with *Enterobius vermicularis*. (12 min.) (2×2 projector). E. H. SADUN AND D. M. MELVIN, CDC, Chamblee.

102. Proteolytic activity in extracts of the esophagus of adults of *Ancylostoma caninum* and the effect of immune serum on this activity. (10 min.) (2×2 projector). R. E. THORSON, Alabama Polytechnic Institute.

103. The effect of extractions of the amphidial glands, excretory glands and esophagus of adults of *Ancylostoma caninum* on the coagulation of dog's blood. (10 min.) (2×2 projector). R. E. THORSON, Alabama Polytechnic Institute.

104. Evaluation of the reliability of the dilution egg count and the direct smear egg count in the quantitative determinations of hookworm infections. (10 min.) (2×2 projector). D. M. MELVIN, E. H. SADUN AND R. HEIMLICH, CDC, Chamblee.

105. The occurrence of a protostrongylid nematode in the meninges of Michigan deer. (10 min.) (2×2 projector). D. L. DEGIUSTI, Wayne University.

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FRIDAY AFTERNOON SESSION, DECEMBER 30, 2:00 P.M., MIRROR ROOM, DINKLER PLAZA, L. O. NOLF, STATE UNIVERSITY OF IOWA, PRESIDING.

*Read*

(Please abide by the time limits)

106. Some nutritional and physiological changes taking place in lambs infected with *Haemonchus contortus, Trichostrongylus colubriformis* and *Nematodirus spathiger*. (12 min.) (2×2 projector). R. F. SHUMARD AND D. F. EVELETH, North Dakota Agricultural Experiment Station.

107. Effect of two rations, differing primarily in protein, carbohydrate and crude fiber content, on experimental haemonchosis in lambs. (10 min.) (Standard projector). K. C. KATES AND G. I. WILSON, USDA.

108. The relation of pasture rotation to acquisition of strongyline nematodes by sheep. (12 min.). N. D. LEVINE AND D. T. CLARK, University of Illinois.

109. Some preliminary observations on the life history of *Amidostomum anseris*, Zeder, 1800. (12 min.) (2×2 projector). A. B. COWAN, Patuxent Research Refuge. (Introduced by C. M. Herman, Patuxent Research Refuge).

110. The role of the skin and lungs in the development of *Nippostrongylus muris*. (12 min.) (Standard projector). D. W. TWOHY, Johns Hopkins University.

111. The morphogenesis of the female reproductive system of *Skrjabinoptera phrynosoma* Schulz, 1927, a gastric nematode of Texas horned toads, *Phrynosoma cornutum*. (10 min.). S. H. LEE, Wayland College.

112. The effect of previous infection on filarial worms transferred from one cotton rat to another. (10 min.) (Standard projector). J. A. SCOTT, E. M. MACDONALD AND L. J. OLSON, University of Texas Medical Branch of Galveston.

113. Infection of white rats with the filarial worms of cotton rats. (10 min.) (Standard projector). L. J. OLSON, J. A. SCOTT AND E. M. MACDONALD, University of Texas Medical Branch at Galveston.

114. Black flies (Simuliidae) as vectors of *Ornithofilaria fallisensis* Anderson,

1954. (12 min.) (2×2 projector). R. C. ANDERSON, Ontario Research Foundation. (Introduced by A. M. FALLIS, Ontario Research Foundation).

115. Current status of intestinal parasitism of man in eastern Kentucky. (5 min.) (Standard projector). F. O. ATCHLEY, E. C. HEMPHILL AND D. W. HUNT, CDC, Prestonburg, Ky.

116. Some problems in parasite control in Southern Brazil. (15 min.) (2×2 projector). J. E. ACKERT, Kansas State College; AND U. F. ROCHA, University of Sao Paulo, Brazil.

117. Some helminthological problems in a zoological park. (12 min.) (Standard projector). B. J. JASKOSKI, Chicago Zoological Park and Loyola University, Chicago.

118. Incidence of poultry parasites under different ecological and geographical conditions in Egypt. (12 min.) (2×2 projector). W. M. REID, University of Georgia.

119. Notes on animal filarial parasites in Malaya, with special reference to *Macaca cynomolgus*. (15 min.). D. L. PRICE, Army Medical Service Graduate School; AND L. A. JACHOWSKI, Naval Medical Research Institute.

*Read by Title Only*

120. The extra-corporeal hatching of the eggs of *Trichuris vulpis*. L. M. YUTUC, University of the Philippines.

121. Cellular factors in acquired immunity to *trichinella spiralis*, as indicated by cortisone treatment of mice. C. M. COKER, University of North Carolina.

122. Further studies on plant-parasitic nematodes maintained in altered oxygen tensions. W. A. FEDER AND J. FELDMESSER, USDA.

123. Further studies on the *in vitro* culture of the larvae of *Ascaris lumbricoides suum*. T. D. PITTS, Loyola University of Los Angeles; AND G. H. BALL, UCLA.

124. On the occurrence of certain high energy phosphate compounds in filariform larvae of *Strongyloides ratti*. C. A. JONES, Veterans Administration Hospital; J. C. SWARTZWELDER, Louisiana State University; AND S. H. ABADIE, Veterans Administration Hospital, New Orleans.

125. On the occurrence of glycogen and phosphate esters in filariform larvae of *Strongyloides ratti*. C. A. JONES, Veterans Administration Hospital; J. C. SWARTZWELDER, Louisiana State University; AND S. H. ABADIE, Veterans Administration Hospital, New Orleans.

126. Parasitologic studies in the Far East. XV. A preliminary survey for intestinal parasitism in southern Formosa. L. P. FRICK AND A. P. MOON, 406th Medical General Laboratory, APO 500, San Francisco, Calif.

127. The treatment of human helminthiases with piperazine citrate. J. C. SWARTZWELDER, J. H. MILLER AND R. W. SAPPENFIELD, Louisiana State University.

128. The early migration and growth of *Nippostrongylus muris* in the rat. D. W. TWOHY, Johns Hopkins University.

129. Effects of sodium borate on swine nodular-worm (*Oesophagostomum dentatum*) infective larvae in soil. J. E. ALICATA, University of Hawaii.

130. Parasites of AMPHIBIA. A. C. WALTON, Knox College.

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## ABSTRACTS

1. **An Intestinal Parasite Survey of the Korean Prisoner of War Camp Located on Koje Island.** M. M. BROOKE, Communicable Disease Center; CLYDE SWARTZWELDER, Louisiana State University; FRED J. PAYNE, Communicable Disease Center; PAUL WEINSTEIN, National Institutes of Health; AND WILLIAM W. FRYE, Louisiana State University.

During the Summer of 1951, the Joint Dysentery Unit of the Armed Forces Epidemiological Board collected stool specimens from 1,726 North Korean prisoners of war on the Island of Koje (Korea). A single normally passed stool per person was preserved in two vials, one containing 10% formalin and the other PVA-fixative. The specimens were examined in the United States by the formalin-ether sedimentation technique (portion in formalin) and iron hematoxylin stained films (portion in PVA-fixative).

A total of 1,682, or 97.5%, were found to be infected with one or more species of intestinal parasites. The most prevalent protozoa and helminths were *Entamoeba histolytica* (33.9%), *Entamoeba coli* (42.9%), *Endolimax nana* (32%), *Iodamoeba butschlii* (10.3%), *Ascaris lumbricoides* (81.3%), *Trichuris trichiura* (77.9%) and hookworm (36.7%).

The prevalence rates for the helminths were quite comparable with those reported in a survey of Korean civilians by Hunter and co-workers (1951) while the rates for the protozoa in the prisoners of war were much higher than in the civilians. The utilization of formalin and PVA-fixative for stool preservation and the formalin-ether sedimentation and the permanently stained films for examination constitutes an efficient method of conducting surveys for intestinal parasitic infections.

2. **Treatment of Asymptomatic Amebiasis with Atabrine in Combination with Carbarsone and with Chiniofon.** TAKASHI SASAKI, Yamanashi Medical Research Institute, Kofu, Japan; DALE E. WYKOFF, Army Medical Service Graduate School; MUNEO YOKOGAWA, Institute of Public Health, Tokyo, Japan; AND LAWRENCE S. RITCHIE, 406th Medical General Laboratory, APO 500, San Francisco, California.

A satisfactory level of efficacy was achieved with atabrine (14 days × 0.3 gram) and carbarsone (10 days × 0.75 gram) for the treatment of asymptomatic intestinal amebiasis, corroborating the conclusion of Radke. In one series of 40 cases, 10 failures (25 percent) were observed, some of which may have been due to failure to take all the drugs. In a second series of 17 cases, no failures were observed, and the nonpathogenic protozoan infections were also extensively eliminated. In two series treated with atabrine and chiniofon, comparable results were obtained against *E. histolytica;* however, results against the nopathogenic amebas were not particularly good, which may indicate a lesser margin of therapeutic efficacy against *E. histolytica.* Adminstration of an iodoquiniline compound could be safely extended beyond the 10-day therapeutic interval used, which is hardly allowable with the arsenical.

3. **Cultivation of *Entamoeba histolytica* from Trophozoites in Fecal Suspensions Kept at 25° C. for 4 days.** MORRIS GOLDMAN AND R. K. CARVER, Communicable Disease Center, Atlanta, Georgia.

Although the cultivation of *E. histolytica* from cysts in feces which have been kept for several days at temperatures lower than 37° C. has been reported many times, the successful cultivation of trophozoites under the same conditions has not been reported, to our knowledge. The present paper describes the recovery by cultivation of *E. histolytica* trophozoites which were kept under laboratory conditions in fecal suspensions up to 4 days at 25° C. The objective of this work is a technique which could be used by diagnostic laboratories to obtain positive cultures from mailed-in stool specimens. Such specimens are frequently loose or watery, and likely to contain the trophozoite stages of amebae almost exclusively.

In a single field trial of our method, 15 purged stools showing amebae by direct examination were cultured immediately, and after being received through the mail 2 days later in a central laboratory. Ten cultures were positive by immediate cultivation and 8 after delayed cultivation.

4. **Measuring the Brightness of Individual Amebae Exposed to Fluorescein-tagged Antibodies.** MORRIS GOLDMAN, Communicable Disease Center, Atlanta, Georgia.

Recent developments in microphotometry provide the instrumentation needed for measuring low intensities of light at the cellular level. Fluorescein-tagged antibody provides a means of staining and visualizing antigens at the cellular level. By combining the two techniques, it has been possible to obtain quantitative data on the degree of fluorescence of individual *Enta-*

*moeba histolytica* organisms after they have been exposed to homologous tagged antiserum. Since the brightness of the amebae is, in part, a reflection of their antigen content, quantitation of the fluorescent reaction is potentially an important technique in studying the biology of the amebae. The equipment used in this work was all obtained from commercial sources and a minimum of special machine work was necessary. It is emphasized that many pitfalls exist in attempting quantitation at this level, and careful controls are needed before interpreting the results.

5. **Virulence Studies with a Strain of *Entamoeba histolytica* Maintained *in vitro* in Liqaid Media.** D. J. TAYLOR AND J. GREENBERG, Laboratory of Tropical Diseases, National Microbiological Institute, National Institutes of Health, Bethesda 14, Md.

It has been possible to grow the NIH strain 200 GP with its multiple bacterial flora for long periods in a medium containing inorganic salts, glucosamine, and rice powder with egg albumin (medium 1), gelatin (medium 22), or trypticase (medium 43) as a source of organic nitrogen.

The virulence for guinea pigs of amebae grown in each of these media has been tested. The percentage of guinea pigs infected and the average degree of infection (ADI) has been compared in each trial with that produced by amebae grown continuously on egg slant (LER).

In 6 trials, amebae from medium 1 infected 60 percent of 32 animals (ADI-1.8); amebae in LER infected 70 percent of 44 animals (ADI-2.2). In 11 trials, amebae grown in medium 22 infected 35 percent of 59 animals (ADI-0.7); amebae grown in LER infected 79 percent of 90 animals (ADI-2.7). In 2 trials, amebae in medium 43 infected 76 percent of 16 animals (ADI-2.5); amebae in LER infected 63 percent of 17 animals (ADI-2.0).

Amebae grown in medium 22 were returned to LER for a number of transfers and were tested for virulence in clean guinea pigs. In 5 trials, 44 percent of 25 animals were infected (ADI-1.2); amebae grown continuously in LER infected 71 percent of 41 animals (ADI-2.2).

Further virulence studies with amebae grown in liquid media were prompted by the significant reduction in percentage of animals infected and the low ADI produced by amebae maintained in medium 22 versus the opposite results obtained, to date, with amebae grown in medium 43.

6. **Blood-Induced Blackhead.** W. C. MCGUIRE, Dr. Salsbury's Laboratories.

Several phases of the life cycle of *Histomonas meleagridis*, the protozoan parasite which causes blackhead (histomoniasis) in turkeys, have been postulated but never proven. No observation or positive proof of its presence in the blood stream of this host has been offered nor has any other plausible hypothesis been advanced for its migration from the site of infection in the ceca to the liver.

Blood-induced blackhead infection has been experimentally produced by transfusing blood from the cecal veins of a diseased donor bird into the wing vein of susceptible recipient turkeys. This procedure produces typical blackhead pathology in the liver without the usual involvement of the gastro-intestinal tract. Atypical pathology was also noted in lungs, kidneys, heart and proventriculus.

Transfusion of heart or wing vein blood from the donor turkey into susceptible turkeys with no pathology resulting, tends to indicate that only the portal circulation contains PROTOZOA capable of producing this infection.

Although no observation of recognizable PROTOZOA were found from fresh or stained samples of the transfused cecal blood, these experiments appear to offer positive—if indirect—proof of protozoan passage from the ceca to the liver through the hepatic-portal circulation.

7. **A Summer Survey of the Blood Parasites of Two *Microtus pennsylvanicus* Populations, Ithaca, N. Y.** S. H. KIRNER, K. R. BARBEHENN AND B. V. TRAVIS, Cornell University.

The seasonal incidence of three haemo-parasites, a trypanosome, a sporozoan, and a bartonella-like organism of the field vole was studied during June to October, 1954 in two collecting areas at Ithaca, N. Y. One area was wet with standing water in the spring, and the other well-drained and dry.

Three significant observations were: (1) sporozoan infections were much higher in the wet field, (2) bartonella infections were higher in the dry field and tended to diminish during the course of the study in both areas, and (3) trypanosome infections tended to remain constant and equal in both populations. No significant effect was observed of the parasites upon vole mortality, nor was there any statistical relationship among parasites in multiple infections, in sex or in age groups of the voles.

8. **Incidence Studies on *Trypanosoma diemyctyli* Tobey, 1906 in *Diemyctylus v. viridescens* (Raf.).** JOHN S. MACKIEWICZ, Cornell University.

From November 14, 1953 to May 18, 1954, 206 male and 144 female Common Newts, *Diemyctylus v. viridescens* (Raf.), were collected at Michigan Creek, Danby, in Tompkins County, N. Y. and examined for the presence of *Trypanosoma diemyctyli* Tobey, 1906. There was a progressive increase in the numbers of trypanosomes per cc. of blood from November to March with a sharp decline in April, when the lowest numbers were recorded. In May there was a steep rise; the highest numbers of trypanosomes per cc. of blood were recorded at this time.

The females exhibited a higher incidence (84%) and degree of infection (mean: 149,985 trypanosomes per cc.) than the males (82%; mean: 144,239 trypanosomes per cc.) The parasites were more prevalent in the weight class between 2.00 and 3.75 grams and generally absent in the lower and higher weight class intervals. The greatest frequency of infected individuals was in the 93 to 96 mm. length class; non-infected newts were found scattered in all the length classes (77 to 116 mm.). The sex, length, and weight differences between infected and non-infected newts were not significant at the five percent level.

No significant differences in coloration or color and distribution of spots could be detected between infected and non-infected newts.

9. **Biochemical Studies on Chicken Macrophages Infected with *Trypanosoma cruzi* (Chagas).** LIONEL G. WARREN, The Johns Hopkins University.

Roller tube cultures of macrophages were prepared from the buffy coat of chicken blood. Cultures were infected with a suspension of parasites obtained from NIH diphasic medium blood-agar cultures of *Trypanosoma cruzi*. The parasite suspension was prepared with heat inactivated chicken serum, or horse serum. Heat inactivated chicken serum, or horse serum, was necessary to prevent a lysis of the culture forms obtained from NIH media. Normal chicken serum (a constituent of the tissue culture medium) lysed culture forms of *T. cruzi* within 8 to 15 minutes after exposure. Normal chicken serum did not destroy the intracellular stages of *T. cruzi* in cultured macrophages. The metabolism of parasitized and non-parasitized macrophages was compared. Parasitized cultures consumed approximately three times as much glucose as non-parasitized cultures during 24 hour periods. In parasitized and normal control cultures, 2 moles of lactic acid were produced for each mole of glucose consumed. No difference has been observed in the development of *T. cruzi* in the presence of high or low concentrations of glucose. In parasitized and non-parasitized cultures the same amino acids appear during the 24 hour incubation periods. Identifiable amino acids appearing were aspartic, glutamic, glycine, and alanine. The pattern of glycolysis and glucose consumption observed indicates that there is no interference by the parasites with the uptake and degradation of glucose. The data obtained do not show a correlation between the increased rate of glucose utilization and the total number of parasites present in the culture.

10. **Growth Factor in Normal Rat Serum for *Trypanosoma lewisi*.** D. R. LINCICOME, Naval Medical Research Institute, Bethesda, Md.

*Trypanosoma lewisi* which is normally parasitic only in the rat can be induced to develop in naturally resistant but nutritionally deficient mice provided normal rat serum is supplied to these hosts intraperitoneally. The trypanosome will survive at best up to a week in the peripheral blood stream of well-fed, naturally resistant mice supplied with rat serum, but do not appear to reproduce. Reproduction of the parasite with accompanying large population increments occurs only in hosts that are metabolically imbalanced. Serial subpassage of the trypanosome through more than 20 mice has been obtained in each of two lines or isolates of trypanosomes without the intercalation of the rat host itself. There has been no evidence that the parasite has lost its ability to live in the rat after 20 passages through this unnatural host. The serum of rats recovered from *T. lewisi* infection does *not* support growth of the parasite in naturally resistant hosts. These data have been interpreted as indication of a growth factor(s) present in the serum of normal rats under the conditions employed. Electrochemical fractionation of rat serum is in progress for determination of the component responsible for the growth of the trypanosome in mice.

11. **Observations on the Immunity to *Trypanosoma equiperdum* Induced by Treatment with Oxophenarsine.** WILLIAM CANTRELL AND GORDON D. BETTS (U. S. Public Health Student Research Scholar), The University of Louisville School of Medicine.

An experiment with mixtures of oxophenarsine-resistant trypanosomes and unmodified trypanosomes suggested that destruction of the unmodified strain with 10 μm. per kg. of oxo-

phenarsine, in some instances, induced a state of acquired immunity to the oxophenarsine-resistant strain during the 14-day period following treatment. Additional experiments in which large numbers of trypanosomes were injected intravenously into rats at different intervals after treatment proved that treatment with 2 μm. per kg. of oxophenarsine induced a rapidly developing immunity (present 24 hours after treatment) of a high degree to the major antigenic type present and that in time this immunity extended to other related but not identical antigenic types. Treatment with 10 μm. per kg. caused a more slowly developing immunity which made its appearance on the 3rd day after treatment. Appropriate control experiments were made to exclude persistence of drug as a cause of "immunity." During these experiments we have observed an instance of spontaneous remission in a rat infected with one of the antigenic variants. These observations adequately explain the behavior of the oxophenarsine-treated mixtures.

This investigation was supported in part by research grant (G 4117) from the National Microbiological Institute, of the National Institutes of Health, Public Health Service).

12. **Comparative Studies on the Physiology of Trichomonad Flagellates.** CLARK P. READ, The Johns Hopkins University.

Washed cells of *Trichomonas vaginalis* (Strain 5A) and *T. gallinae* (Strain W-S) have been compared in ability to metabolize anaerobically glucose, galactose, fructose, mannose, maltose, sucrose, trehalose, lactose, starch, glycogen, citrate, isocitrate, alpha-ketoglutarate, succinate, fumarate, malate, and beta-hydroxybutyrate. The two species are somewhat similar in capacity to metabolize sugars but differ sharply in pH optima (*T. vaginalis* pH 6.0; *T. gallinae* pH 7.0). The two species are quite unlike in that *T. vaginalis* is apparently incapable of oxidizing the intermediates of the tricarboxylic acid cycle and beta-hydroxybutyrate, whereas *T. gallinae* oxidizes all of them.

Both species produce acid(s), carbon dioxide, and hydrogen as end products of anaerobic carbohydrate dissimilation. Data on quantitative differences in these products will be presented.

The effects of inhibitors on the carbohydrate metabolism of the two species have been studied. Inhibitors used include phenyl-mercuric-acetate, dinitrophenol, fluoroacetate, glyceraldehyde, phlorizin, malonate, carbarsone, fluoride, iodoacetate, arsenate, arsenite, ethylene-diamine-tetracetate, hydroxyquinoline, cyanide, and azide. The differences in susceptibility of the two species will be discussed.

13. **The Effect of Cysteine on Growth of *Trichomonas vaginalis*.** B. F. SMITH, Spelman College.

The effect of cysteine on the growth of *Trichomonas vaginalis* was observed on the 5A strain of this organism, which has been maintained on CPLM medium (Johnson and Trussell 1943) since 1953. The assay procedure involved the omission of cysteine from a complete amino acid medium and subsequent measurement of population density by cell count as the criterion of growth. The complete amino acid medium in which luxuriant growth was obtained contained nineteen amino acids, ribonucleic acid, and five percent human serum as the nitrogen sources. Culture tubes containing the complete medium and the cysteine-less medium were each inoculated with approximately 800,000 organisms. One half of these tubes was placed in a desiccator and evacuated approximately to minus 760 mm. of pressure with the use of a vacuum pump. This was performed in order to create an essentially anaerobic environment. Tubes of the other half were not evacuated and were grown aerobically. Both the evacuated and the non-evacuated cultures were incubated at 37° C. for 48 hours and maintained through two serial transfers.

The decrease in population in the 2nd serial transfers of the non-evacuated, cysteine-less and the evacuated cysteine-less cultures were 76% and 98% from their respective cysteine-containing controls. These results indicate that cysteine, which is routinely used in *T. vaginalis* media as a reductant, is also necessary for growth of this organism under both anaerobic and aerobic conditions.

14. **Experimental Treatment of Bovine Trichomoniasis in Bulls with Acriflavine and other Agents.** PAUL R. FITZGERALD, Animal Disease and Parasite Research Branch, ARS, USDA; AND JOSEPH THORNE, A. EARL JOHNSON, AND DATUS M. HAMMOND, Utah State Agricultural College.

In an effort to find a simpler and more effective method of treating bulls several materials and methods of application were tried. Some agents were applied systemically, but most treatments involved applications of ointments or of fluid douches to the penis and prepuce of in-

fected bulls, with or without anesthesia. Enheptin[R], Carlendacide, Fumagillin, and a cream containing gentian violet were all ineffective against *Trichomonas foetus in vivo*.

Acriflavine ointment at 0.5 percent was not effective when it was injected into the prepuce with a grease gun daily for five days, although one bull was negative for a period of one month following treatment. Acriflavine ointment massaged into the mucosa of the penis and prepuce while the bull was under pudendal anesthesia was effective every time it was used. However, 1 percent ointment caused severe reactions, involving inflammation and edema followed by necrosis and adhesions. With reduction of the concentration of acriflavine to 0.25 percent only a slight reaction occurred. All of six bulls treated with one application of this concentration of acriflavine were cured. The results indicate that further trial of 0.25 percent acriflavine is justified.

15. **Experimental Infections of the Bovine Genital Tract with Trichomonads from Swine.** PAUL R. FITZGERALD, Animal Disease and Parasite Research Branch, ARS, USDA; AND A. EARL JOHNSON, JOSEPH THORNE, AND DATUS M. HAMMOND, Utah State Agricultural College.

When trichomonads from cultures from the nose, stomach or cecum of swine were inoculated intracervically with semen into six virgin heifers at estrus, infections developed which persisted 35 to 76 days. Similar inoculations with *T. foetus* from cultures and from preputial samples resulted in infections lasting 18 and 100 days, respectively. All of these heifers returned to estrus. Two heifers not in estrus inoculated intravaginally with trichomonads in nasal washings of swine or from cultures from the stomach of swine became infected, the infections persisting 29 and 38 days respectively. No infection resulted when a heifer was similarly inoculated with swine cecal contents, nor when a bull was bred to the heifer infected with swine stomach trichomonads. Five pregnant heifers were inoculated with cultures of trichomonads. In one four-months pregnant heifer inoculated with swine cecal trichomonads into the uterus by laparotomy an infection resulted, with an abortion 20 days after inoculation; vaginal samples were positive 20 to 68 days after inoculation. Three other heifers similarly inoculated with swine nasal or stomach trichomonads or *T. foetus* showed no demonstrable infection; two completed normal pregnancies and one returned to estrus. A heifer pregnant 34 days inoculated with swine nasal trichomonads returned to estrus and vaginal samples were positive 4 to 56 days after inoculation. The results show that trichomonads from swine are able to become established in the genital tract of heifers and that such infections may be associated with infertility or abortion.

16. **Further observations on occurrence of *Hepatozoon* in gray squirrels** (*Sciurus carolinensis*). CARLTON M. HERMAN, Patuxent Research Refuge, U. S. Fish and Wildlife Service, Laurel, Maryland; AND GORDON M. CLARK, University of Maryland.

This parasite, previously reported in the United States only from *Rattus norvegicus* in several surveys in eastern cities and, in one case, from California ground squirrels, has recently been observed in the gray squirrel in Maryland and Washington, D. C. (Herman and Price, 1955). These earlier studies of squirrels were confined to the vicinity of Baltimore and Washington. Subsequent examination of blood samples from squirrels of eight areas in Maryland indicates that *Hepatozoon* occurs in gray squirrels throughout this state. *Hepatozoon*-positive specimens have also been obtained from West Virginia.

The prevalence of this protozoan in gray squirrels has, in all areas thus far studied, been found to be exceedingly high. Parasites were readily found by direct blood smear examination, and the employment of a concentration technique described by Herman and Price (1955) has revealed infection in nearly every squirrel so tested. All of a total of 12 squirrels, examined at 3–6 weeks of age, (during August and September), exhibited a parasitemia. One of these, collected in 1954, continued to show a parasitemia in all smears made during the succeeding year.

Because of the morphological similarity between some stages of *Hepatozoon* and *Toxoplasma*, blood sera from a number of *Hepatozoon*-infected squirrels were examined by the dye-test for *Toxoplasma* in the laboratory of Dr. L. Jacobs at the National Institutes of Health. No cross reactions were evident.

The currently known prevalence of *Hepatozoon* in gray squirrels suggests a possible broad geographical distribution in this host. If this is so, materials should be readily available for studies on the biology and taxonomic positions of this genus.

17. **The Development of *Hepatozoon sciuris* Coles, 1914 in Two Parasitic Laelaptid Mites.** GORDON MARSTON CLARK, Department of Zoology, University of Maryland.

Gray squirrels in the Washington, D. C. and eastern Maryland area show a high incidence of infection with *Hepatozoon sciuris*. Possible intermediate hosts of this protozoan have been investigated. *Orchopeas howardii* (Baker, 1895), a flea, and two species of parasitic mites, *Haemolaelaps casalis* (Berlese, 1887) and *Haemogamasus barberi* Ewing, 1925, are commonly found in squirrel nests in this area. *O. howardii* has not been found to carry the parasite. Development of *H. sciuris* has been observed in *H. barberi* and in a species, *Echinolaelaps echidninus* (Berlese, 1887), that is easily cultured in the laboratory.

*E. echidninus* was artificially infected using the blood feeding technique of Cross (1954). Development of the parasites was checked at regular intervals by observing crushed mites in physiological saline with the phase microscope. Stained smears and serial sections of infected mites were also utilized.

*H. sciuris* has been found to reach the infective sporozoite stage in the body cavity of *E. echidninus* in approximately 30 days. Similar forms are commonly found in sections of naturally infected *H. barberi* taken from squirrel nest boxes in the field.

18. **Sulfaquinoxaline and Sulfamerazine in the Treatment of Experimental Infections with *Eimeria bovis* in Calves.** DATUS M. HAMMOND, J. LEGRANDE SHUPE, A. EARL JOHNSON, JOSEPH THORNE, Utah State Agricultural College; AND PAUL R. FITZGERALD, Animal Disease and Parasite Research Branch, ARS, USDA.

In six tests, each with 12 calves, all were inoculated with 0.5 million to 1 million oocysts, chiefly *Eimeria bovis*, in one to six doses. In each test one group was untreated and the remaining ones were treated.

In three tests sulfaquinoxaline was given at 1 grain per pound of body weight for one day and 0.5 grain for three days. Treatment was begun in some groups at the time of first inoculation and 21 days later, in other groups 7 and 21 days after inoculation, and in the remaining treated groups 17 days after inoculation. There was little or no protection against coccidiosis.

In three tests sulfamerazine or sulfaquinoxaline was given at 1 grain per pound of body weight for two days and 0.5 grain for two days. In one test, treatment with sulfamerazine was begun 13 days after inoculation in one group, and in another group one day before inoculation and repeated 14 days later. In a second test, treatment with sulfamerazine was begun 13 days after inoculation in one group and 18 in another. In a third test, sulfamerazine or sulfaquinoxaline was given 13 days after inoculation. All groups treated beginning 13 days after inoculation showed slight or no symptoms and discharged few oocysts; sulfaquinoxaline was somewhat less effective than sulfamerazine. Treatment beginning 18 days after inoculation was ineffective. The stages of *E. bovis* which occur 13 to 17 days after inoculation apparently are most susceptible to these drugs. (This research was supported in part by a grant to the Utah Agricultural Experiment Station from Sharp and Dohme Company.)

19. **Size of the Oocyst of *Eimeria brunetti* and *E. necatrix*.** ELERY R. BECKER, WALTER H. PATTILLO, JOHN N. FARMER AND WILLA MAE VAN DOORNINCK, Iowa State College.

*Eimeria brunetti*. The overall range in size of a total of 1550 unsporulated oocysts from seven chickens was 13.8–33.7 microns for length and 12.4–28.3 microns for width. The average of the means of the 19 samples measured was 23.4 microns by 19.7 microns.

*Eimeria necatrix*. Measurements on 3300 unsporulated oocysts from four chickens available at the time of writing showed an overall range in size of 13.1–26.8 microns for length and 10.7–22.2 microns for width. The average of the means of the 48 samples measured was 19.3 microns by 16.5 microns.

The effects of day of the infection on which the samples of oocysts are discharged and the individuality of the hosts will be discussed as the measurements are presented.

20. **The Effect of Cecal Coccidiosis on the Motility of the Digestive Tract of the Domestic Fowl.** C. S. SCHILDT, Henderson State Teachers College; AND C. A. HERRICK, University of Wisconsin.

During the course of infection caused by *Eimeria tenella* the motility of the alimentary tract of chickens was disturbed.

Feed was retained in the crop of most infected chickens during the fifth day after infection and if not retained the passage of feed was delayed. Finely divided charcoal and Evans blue were used as indicators. Indicators injected into the gizzard and small intestine appeared in the feces as soon in infected birds as in the normal chickens.

A modified Trendelenberg apparatus was designed to record, by means of a kymograph,

the *in vitro* activity of the cecal pouches and small intestine. The same apparatus was utilized in recording the *in vivo* activity of the crop.

The *in vitro* activity of the cecal pouches ceased at the end of the fourth day following infection, and normal function did not return for a period of about three weeks.

A decrease in crop activity was evidenced on the third day after infection. On the fifth day after infection the crop had ceased to function in most cases. A period of about two weeks elapsed before crop activity returned to normal.

21. **A Comparative Study of the Effects of Cecal Coccidia on the Ability of Plymouth Rock and Leghorn Chickens to Do Muscular Work.** LAURENCE LEVINE, Wayne University; AND CHESTER A. HERRICK, University of Wisconsin.

The cecal coccidia, *Eimeria tenella*, reduced the ability of chickens to do muscular work but the effects were more pronounced in Single Comb White Leghorns than in Barred Plymouth Rocks.

In both control and infected birds the significantly heavier muscles of Plymouth Rocks did more work per contraction and more total work during a three minute period than did those of Leghorns of the same age. The muscles of the Leghorns, however, did more work per contraction per gram than did those of Plymouth Rocks. The same was true of infected Leghorns when stimulated directly, but not when stimulated through the nerves.

Preliminary experiments on the contractile proteins of the leg muscles showed that both breeds had the same actomyosin content per gram of muscle but the ratio of actin to myosin was 1 to 8 in the Plymouth Rocks and 1 to 24 in the Leghorns.

22. **The Effect of Nicarbazin on avian coccidia.** ASHTON C. CUCKLER AND CHRISTINE M. MALANGA, Merck Institute for Therapeutic Research, Rahway, New Jersey.

Nicarbazin, an equimolecular complex of 4,4′ dinitrocarbanilide and 2-hydroxy-4,6-dimethylpyrimidine, has recently been described (Science, *122:* 244–245, 1955) as an anticoccidial agent for chickens. This paper summarizes data on the anticoccidial effectiveness of nicarbazin. It also presents the results of studies on the development of immunity following the use of nicarbazin. In addition, data are presented on the effect of nicarbazin on the developmental stages of *Eimeria tenella* and *Eimeria necatrix*. These studies show that nicarbazin effectively prevents coccidiosis and permits the development of a high degree of immunity to re-infection. Histopathological and other studies show that the merozoites of the second generation schizonts are suppressed by nicarbazin. In conclusion, nicarbazin medication (0.0125%) greatly reduces the damaging pathological effects of coccidiosis without interfering with the development of acquired immunity.

23. **Comparative Susceptibility of Erythrocytes from Mammals and Birds to Infection by Four Species of Avian Malaria.** R. BARCLAY MCGHEE, University of Georgia.

The erythrocytes of various species of animals, including those of several mammals, are susceptible to infection by *Plasmodium lophurae*. To determine whether this cell susceptibility is peculiar to *P. lophurae* or is a generalized phenomenon, three additional species of avian malaria were studied, using certain of the same kinds of cells previously exposed to *P. lophurae*. When parasitemias (*P. gallinaceum* and *P. circumflexum* in chick embryos and *P. cathemerium* in duck embryos) had reached suitable levels the infected embryos were intravenously injected with washed erythrocytes from pigeons, ducks, canaries, mice, rabbits, adult and baby rats. Blood films were made 4 and 24 hours afterwards and the amount of infection determined. Bird erythrocytes were susceptible to all four malarias, but with considerable differences in the rate of penetration. An arbitrary designation of complete, moderate and slight susceptibility was given, depending on the percentage of available merozoites penetrating cells. Erythrocytes of the pigeon, canary and duck were only slightly susceptible to *P. gallinaceum*, moderately so to *P. cathemerium* and completely so to *P. circumflexum* and *P. lophurae*. There was no penetration of mammalian cells by *P. gallinaceum*, slight penetration of baby rat cells by *P. circumflexum* and some invasion of adult rat erythrocytes by *P. cathemerium*. In all instances, once invasion had taken place, normal development ensued, indicating that the mechanism of resistance is elsewhere than in the internal medium.

24. **Further Observations on the Transmission and Development of *Leucocytozoon simondi* M. & L.** A. M. FALLIS, R. C. ANDERSON, G. F. BENNETT, Ontario Research Foundation, Toronto, Canada.

Development of *Leucocytozoon simondi* occurs in species of *Eusimulium* and in *Simulium rugglesi* which species are important vectors of this parasite as shown by the collections of

gorged flies that were taken from ducks. Species of *Eusimulium* were taken most frequently from ducks in the early part of the black fly season and *S. rugglesi* during part of June and July. Small oocysts (ca. 11 microns) have been observed on and below the stomach wall of *S. rugglesi*. This development appears to be completed in a few days. Relatively few sporozoites are produced in each oocyst. Sporozoites, similar to those reported previously, were demonstrated in the salivary glands of the infected flies. Further observations on the asexual development of the parasite in ducks are presented also.

25. **Relationship of size of inoculum to time to death in mice infected with *Toxoplasma gondii*.** DON E. EYLES AND NELL COLEMAN, National Institutes of Health, Memphis, Tennessee.

The relationship between time to death and size of inoculum for *Toxoplasma gondii* is well described by a linear regression of time to death on the logarithm of the number of free organisms injected intraperitoneally into white mice. When organisms were suspended in serum the relationship followed the equation, $Y = 8.91 - 0.67\ X$; whereas, when the suspending agent was physiologic saline the relationship was found to be, $Y = 12.05 - 1.27\ X$. The difference between the equations most probably represented a deleterious effect of the saline solution on survival of the parasites.

The ID50 inoculum described in terms of the number of free parasites injected was found to be approximately one for organisms suspended in serum, thus indicating that a very small number of organisms, if not one, may initiate a fatal infection. When the organisms were suspended in saline the ID50 inoculum was approximately 30, another indication of deleterious effect. The data showed that mice receiving the minimal inocula mostly died by the eleventh day, and that the minimal lethal dose was close to, if not identical to, the minimal infective dose.

It was suggested that the regression equations might provide a practical means of estimating the number of organisms present in experimental tissue inocula containing unknown numbers of parasites of the same strain. It was also suggested that the regression relationships might be used as measures of virulence in the comparison of various strains of *Toxoplasma*, and that the slope of the curve in the case of organisms suspended in serum might be indicative of the multiplicative capacity of the parasite in the host under study.

26. **Immunity in Murine Toxoplasmosis.** LEON JACOBS AND MARJORIE L. MELTON, U. S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Microbiological Institute, Laboratory of Tropical Diseases, Bethesda, Maryland.

Mice which survive infection with the relatively avirulent strain of *Toxoplasma*, 113-CE, generally succumb to challenge with the highly virulent RH strain. While there are occasional survivors of challenge, the most salient evidence of an immune response is the prolongation in survival time. The degree of immune response has been shown to vary with time since the first infection. Five-month-old mice which had been left uninfected, or had been infected intraperitoneally 1, 2, or 3 months previously with 1,000 parasites of strain 113-CE, were all challenged at the same time with 100 RH parasites. The previously uninfected mice lived an average of 7.4 days (range 7–8) after challenge. Those infected 1, 2, or 3 months previously survived on the average, 18.2, 12.7, and 10.2 days respectively after RH challenge. The respective ranges in survival time were as follows: 13–28, 8–20, and 8–14. Essentially the same results were obtained in four similar experiments, although the higher immunity of the most recently infected mice was not always so strikingly manifest and the ranges in survival time of control and immunized mice overlapped. There was no evidence obtained, by subinoculations of brain from immunized mice, that the immunity was related to premunition.

27. **Parasitological and Serological Studies on the Prevalence of Canine Toxoplasmosis in Memphis, Tennessee.** COLVIN L. GIBSON AND JOHN R. JUMPER, National Institutes of Health, National Microbiological Institute, Laboratory of Tropical Diseases, 874 Union Avenue, Memphis, Tennessee.

During 1954, Sabin-Feldman dye tests for anti-toxoplasmic antibodies were performed on 137 dogs obtained at random from the Memphis dog pound. Emulsified tissues from these dogs (brain, liver, spleen) were also inoculated intraperitoneally into white mice in an attempt to isolate *Toxoplasma* organisms. Two additional blind passages were made (in mice) from each series, and each mouse was given 5 mg. of cortisone acetate intramuscularly to enhance susceptibility to *Toxoplasma*. No infections were detected in this group of dogs, of which 30 percent had low antibody titers (less than 1 : 16) and 15 percent had titers of 1 : 16 or above.

During 1955, 543 dogs were screened by the dye test procedure; 72 (13 percent) had titers

of 1:16 or higher. Of these latter, 44 were inoculated (brain only) into mice by the procedures described above. Two *Toxoplasma* infections were thus detected in this group of dogs.

Analysis of dye tests on the 680 dogs in this study did not detect a difference between the sexes as to prevalence of positive titers or high titers; nor did we find a difference in prevalence of positive titers between urban and rural dogs. Our data indicate that adult dogs have a significantly higher proportion of titers of 1:16 or above than do juveniles; however, our test procedures do not permit any conclusions as to whether this difference likewise applies to lower titers.

28. **The Therapeutic Value of Fumagillin in the Control of *Nosema apis* Zander in the Honey Bee.** H. E. Cmejla, Abbott Laboratories, North Chicago, Illinois; C. A. Herrick, The University of Wisconsin; and C. L. Farrar, The University of Wisconsin and Entomology Research Branch, Agricultural Research Service, United States Department of Agriculture, Madison, Wisconsin.

Studies on the activity of the antibiotic, fumagillin, against *Nosema apis* were conducted in both the laboratory and under practical apiary conditions.

The laboratory studies were carried on with worker bees taken from a stock colony, maintained in wooden cages, and provided with glass vials containing water and sugar syrup as food. Infections with *Nosema* caused a reduction of from 25 to 58 percent in the length of life of workers. However, infected workers treated with fumagillin lived as long as uninfected controls. Under the conditions in these studies, the infection was not completely eliminated but fumagillin gave a high degree of control at concentrations of 25 mg. fumagillin activity per liter of syrup and above. Even concentrations as low as 10 mg. fumagillin activity per liter afforded some reduction in parasitism.

Field experiments, carried on with colonies established with package bees, demonstrated that this disease can be controlled effectively by making fumagillin-medicated syrup available to the colony during the first three weeks of colony development. Treated colonies were superior to the untreated as evidenced by fewer queen losses, quantitatively and qualitatively better brood production, and larger numbers of bees present with impressive differences in build-up.

29. **Cytochemical Observations on Cultured Macrophages Infected with *Leishmania donovani*.** Clark P. Read and Patricia Chang, The Johns Hopkins University.

Chicken buffy coat cells were cultured on cover glasses, three of which were placed in a flattened tube (Fisher Agarslant) with 10 to 12 drops of 50% inactivated chicken serum—50% Gey's salt solution. Surface tension holds the cover glasses on the wall of the tube and the preparation was readily incubated on the usual roller drum assembly. The medium was changed 3 times during a 5 day period, and on the 5th day the cells were infected with washed *Leishmania donovani* from N.I.H. diphasic cultures. Cover slips were removed from the tubes at 24 hour intervals and cytochemical studies carried out on cover glass preparations. Uninfected cultures were studied simultaneously.

Infected cells contain practically no fat droplets although uninfected cells are rich in fat. These droplets are sudanophilic, osmophilic, and are not strongly stained by Baker's phospholipid reagent. Infected cells show no increase in esterase or alkaline phosphatase activity when compared with uninfected controls.

Infected cells showed a marked increase in production of material which is stained by Best's carmine; this material is probably mucoid in character since it is not affected by treatment with salivary amylase.

The methods used herein for cultivation offer many advantages in obtaining cover glass preparations for cytochemical study of a number of parasites which invade cells.

30. **Repeated enhancement in virulence of a strain of *Toxoplasma* by passage in mice.** Marjorie L. Melton and Leon Jacobs, U. S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Microbiological Institute, Laboratory of Tropical Medicine, Bethesda, Maryland.

Increase in virulence of a strain (113-IP) of *Toxoplasma gondii* passaged in mice, and maintenance of low virulence in a chick-embryo-passaged line (113-CE) has been reported by Jacobs and Melton. It was of interest to determine whether the modification in the mouse-passaged line was a fortuitous finding or could be repeated. For this purpose, another line (632) was established by inoculating mice intraperitoneally with 113-CE toxoplasmas. Transfers were made twice weekly. All mice survived the first 4 passages, and deaths occurred regularly only after the tenth passage. Production of peritoneal exudates was rare until after the 12 passages, requiring transfer by inoculation of tissue suspensions. Peritoneal exudates con-

tained relatively few parasites until the 29th passage, after which the toxoplasmas were numerous. After 39 passages, the virulence had increased so that inocula of 10, 100, and 1,000 parasites killed mice in an average of 8.3, 7.5, and 7 days respectively. Together with observations on virulence of strains isolated from spontaneous infections in animals (Sabin, Armstrong and MacMurray, de Rodaniche) and therefore maintained in mice, these findings emphasize the changing character of mouse-passaged parasites.

31. **Life-Cycle of** *Diplostomum (baeri?)* **(Trematoda: Strigeida).** GLENN L. HOFFMAN, University of North Dakota.

In 1954 a new *Diplostomulum* was found in a tumor-like "cyst" on the brain of the stickleback, *Eucalia inconstans* [Hoffman, J. Parasit. **40** (5, Sect. 2): 32]. Adults were obtained by force-feeding newly hatched unfed chicks and also by injecting metacercariae into the visceral cavity of same. The adult is tentatively identified as *D. baeri* (Dubois, Georges, 1954, personal communication). Many viable eggs were recovered from worms from both the intestine and the visceral cavity. The eggs hatched in about 12 days and the miracidia were studied.

Seventeen of 70 *Stagnicola palustris* and *S. p. elodes* were experimentally infected. At about 30 days large filiform mother sporocysts were present in the visceral cavity of the snail, and mother and daughter sporocysts were present in the digestive gland. The filiform, cercaria-producing, daughter sporocysts were much smaller. The cercaria is very similar to *Cercaria scudderi* Olivier, 1941, but differs in several respects.

Diplostomulum-free stickleback were readily infected with the cercariae. At 7 days the body was not yet shaped like the mature diplostomulum, but the beginning of the hold-fast could be seen as a shallow but definite depression. At 9 days the hind-body was well formed, giving the metacercaria the typical appearance of the mature diplostomulum. At 11 days the "calcareous" corpuscles of the reserve excretory system were not present but at 14 days they were approximately half formed (about 4 microns in diameter), and at 18 days they could not be differentiated from those of the fully mature metacercaria.

Studies on the development of the tumor-like "cyst" and the natural definitive host are in progress.

Supported in part by research grant B-855 from the Institute of Neurological Diseases and Blindness of the National Institutes of Health, Public Health Service.

32. **Studies on the Life-Cycle and Development of** *Crassiphiala bulboglossa* **(Trematoda: Strigeida).** GLENN L. HOFFMAN, University of North Dakota.

Eggs were recovered from adult *Crassiphiala bulboglossa* from the kingfisher, *Ceryle alcyon*, and the resultant miracidia studied. Snails (*Heliosoma anceps*) were successfully infected, and a tangled mass of filiform sporocysts developed in the digestive gland. Furcocercous cercariae emerged from the snails at 33 days. Fishes, *Pimephales p. promelas*, *Fundulus diaphanus menona*, and *Eucalia inconstans*, were experimentally infected.

The miracidium is very similar to that of *Uvulifer ambloplitis*, a related species, as described by Hunter and Hunter, 1934 (23 Ann. Rep. N. York State Cons. Dept., [1933] Suppl.: 245–254). The cercaria is likewise very similar to that of *U. ambloplitis* (=*Cercaria bessiae*) as described by Cort and Brooks, 1928 (Tr. Am. Micr. Soc. **47**: 179–221) but differs in size of tail stem and furcae, number of flame cells, lack of unpigmented eye spots and presence of body flagella. The cercariae readily penetrate the afore-mentioned fish and develop into typical *"Neascus bulboglossa"* cysts in the skin of *Pimephales p. promelas*, and into non-pigmented but otherwise typical metacercariae in *Fundulus diaphanus menona*. Development of the metacercaria is slow in *Eucalia inconstans*, however, and the parasite never becomes a fully developed metacercaria and finally dies in 2 to 3 months. During normal development the parasite becomes *"Neascus"* shaped at about 16 days and the cyst of parasite origin is formed at about 20 days. Structures which are apparently cystogenous glands can be seen in cross sections just before the parasite cyst is formed but not afterwards.

33. **Growth and Development of** *Schistosoma mansoni* **in Mice.** MYRON G. RADKE, MORRIS D. SCHNEIDER, AND DANNIE G. HOUGHTALING, SU 3004, Third Army Area Medical Laboratory, Fort McPherson, Georgia.

The rate of growth and development of *Schistosoma mansoni* experimentally propagated in more than 100 mice has been determined. The growth of male schistosomes was unrestrained during the period of infection studied, but females appeared restricted in their growth. Total solids and nitrogen content were determined for male, female and bisexual worms.

34. **The Structure and Source of the Pericercarial Envelope (CHR) of** *Schistosoma*

*mansoni*. F. J. KRUIDENIER, University of Illinois, AND M. A. STIREWALT, Naval Medical Research Institute.

The formation *in vitro of* a pericercarial membrane (CHR) around cercariae of *Schistosoma mansoni* exposed to the sera from schistosome-infected hosts has been variously demonstrated. This phenomenon has been associated with the agglutination of these cercariae in sera from infected hosts. Knowledge of the detailed structure of the membrane and recognition of its source promote a more complete understanding of its mechanics.

A series of normally emerged cercariae was fixed from aquarium water; after treatment with normal mouse serum; and after treatment with mouse antiserum for 1 minute, 3 minutes 20 minutes, 1 hour, and 20 hours. Cercariae were fixed in the mammalian isotonic-osmic acid solutions recommended by Rhodin, sectioned on the Sjöstrand ultra-microtome, and sections examined with the RCA electron microscope. The progressive development of a fine extra-cuticular film present around recently emerged cercariae fixed from water appears to produce the pericercarial envelope (CHR) by combination with antiserum elements.

Histochemical analysis demonstrates that the fine film surrounding normally emerged cercariae is formed from secretions of the "penetration gland" complex. Initial discharge of these glands is from immature cercariae prior to their emergence from sporocysts. The film forms at this time. Discharges by cercariae subsequent to their emergence from snail tissues do not appear to contribute to the pericercarial film nor aid in the formation of the pericercarial envelope (CHR).

35. **Penetration of Host Skin by Cercariae of** *Schistosoma mansoni*. **I. Observed Entry into Skin of Mouse, Hamster, Rat, Monkey and Man.** M. A. STIREWALT, Naval Medical Research Institute, Bethesda, Maryland.

Entry into various skin areas of the hosts was observed both with reflected light and the dissecting microscope and with transmitted light and the 16 mm. objective of the compound microscope. Variations in the ability of cercariae to enter, in the site of entry, and in times spent in exploration and actual entry were noted according to the condition of the skin with reference to hair, follicular orifices, wrinkles, scaliness, etc. For instance, adult haired (C3H) mouse tail and adult monkey body skins were entered by 88% to 91% of the cercariae applied, while 5 day old rat body skin was entered by only 34%. The entry sites of choice in human body, monkey body, adult hairless mouse body, 1–2 day old mouse body, 4–5 day old mouse body, and 5 day old rat body skins were the walls of skin wrinkles. Follicular orifices were used by more than half the cercariae entering adult haired mouse tail, hairless mouse tail and ear, and hamster body skins. Time spent in exploration was least in human skin and greatest in 5 day old rat body skin. Actual entry was fastest into 1–2 day old mouse body and adult hairless mouse body and ear skins and slowest into hamster and human body skins.

36. **Serologic Reactions in** *Schistosoma mansoni* **Infections: Ionographic Fractionation of Sera of Mice with Progressive Disease.** A. S. EVANS AND M. A. STIREWALT, Naval Medical Research Institute, Bethesda, Maryland.

Seventy mice of the same age were divided into two equal groups. One group was exposed to 50 cercariae each of *Schistosoma mansoni;* the other, unexposed, was maintained under identical conditions as controls. Mice from each group were bled after 5, 12, 20, 26, and 56 weeks, total serum protein was determined by a microkjeldahl technique, and the serum fractionated by paper electrophoresis using 0.1M veronal buffer, pH 8.6, at 2.7 or 3.1 volts/cm. for 26 or 24 hours respectively, at 5–8° C. The various components were identified by analogy with similarly separated human serum fractions and their movement relative to that of the mouse albumin peak. In the early disease, before deposition of eggs in the liver and migration of the worms to the mesenteric vessels, no notable changes in the serum protein fractions were observed. However, with progressive disease, disturbance of the protein or lipoprotein synthesis mechanisms was evidenced chemically by an increase in total proteins referable by ionographic determination directly to an absolute increase in the gamma-beta globulin regions. Decrease in albumin due to liver damage was relative and the absolute concentration of this component remained constant within biological limits. The previously reported disappearence of the alpha$_1$ fraction in old schistosome infections appeared to be, at least in part, gerontic.

37. **Intradermal Reactions to** *Schistosoma japonicum* **and** *S. mansoni* **Antigens in Schistosome Dermatitis Cases.** H. F. HSÜ, State University of Iowa; AND D. J. AMEEL, Kansas State College.

Forty-seven recent or old cases of schistosome dermatitis in the vicinity of Lake Douglas, Michigan, were intradermally tested with the worm antigen of *Schistosoma japonicum* and cercarial antigen of *S. mansoni*. Three cases were positive to *S. japonicum* antigen and one to *S. mansoni* antigen. The antigens were at the same time tested intradermally on 20 cases without any history of schistosome dermatitis. All of them showed negative reactions.

It was concluded that schistosome dermatitis cases usually do not give positive intradermal reactions to the antigens made from the human schistosome, *S. japonicum* or *S. mansoni*. However, occasionally a positive reaction does occur.

The present studies were made at the University of Michigan Biological Station and were supported by a research grant, E. -939, P.T.M. (1), National Microbiological Institute, the National Institutes of Health, U. S. Public Health Service.

38. **Immunologically Reactive Substance from** *Schistosoma mansoni*. MORRIS D. SCHNEIDER, MYRON G. RADKE, AND MARION T. COLEMAN, SU 3004, Third Army Area Medical Laboratory, Fort McPherson, Georgia.

Information of the chemical composition, metabolism and immunochemical characteristics of schistosome worms is meager. An exploratory study of the immunochemistry of adult *Schistosoma mansoni* has been undertaken. A nondialyzable cell-free aqueous material has been derived by chemical means from *Schistosoma mansoni*. The isolated substance 1) represents 10 to 20 percent of the dry weight of the worms, 2) is chemically complex, containing 7 to 9 percent organically-bound nitrogen, carbohydrate and nucleoprotein components, and 3) possesses a relatively heat-stable antigen which is immunogenic in the rabbit and reacts in the complement-fixation test with serum of human *Schistosoma mansoni* disease. Data pertaining to this phase of work will be reported.

39. **Studies on Schistosomiasis. XII. Results of Experiments on Screening Potential Protective Ointments on Mice against the Cercariae of** *Schistosoma mansoni*. G. W. HUNTER, III, Fourth Army Area Medical Laboratory, Fort Sam Houston, Texas; H. A. KEMP; H. E. SMALLEY; O. P. WILKINS AND C. DIXON, Baylor University.

During the second year of screening potential protective ointments against schistosomiasis using white mice and cercariae of *Schistosoma mansoni* 127 compounds were tested. Ten ointments gave complete protection following the Four Hour Immersion Test: Actamer (10%) in Silicote; Actamer (5%), Hexachlorophene (3%), Benzyl benzoate (20%) in wool fat; Benzyl benzoate (20%) and Abbott's Covicone with repellents (E4856); Hexachlorophene (10%) in wool fat; Hexachlorophene (5%) in wool fat; Hexachlorophene (5%), Benzyl benzoate (20%), copper stearate and copper oleate in ether; Hexachlorophene (5%,) copper stearate (40%,) with copper oleate in ether; Hexachlorophene (3%) and Abbott's Covicone with repellents (E4856); Hexachlorophene (10%), olive oil (10%) in petrolatum; and Hexachlorophene (3%) in Pro-derma.

Eight others provided over 99 percent relative protection while 15 gave 90–99 percent and 94 ointments yielded less than 90 percent.

Twenty-nine ointments were subjected to *in vitro* tests. Semiqualitative toxicity tests consisting of 15 topical applications during a three weeks period were run on 14 of the best ointments. The five best ointments were selected for further study.

40. **Chromosomes and Sex Determination in** *Schistosomatium douthitti* **(Trematoda: Schistosomatidae).** ROBERT B. SHORT, Florida State University.

Chromosomes were studied in sectioned and squashed cercarial embryos and in a few germinal cells of both sexes within snails previously infected with one miracidium each. The miracidia were obtained from bisexual infections in mice and the sex of each infection was determined by mouse exposure.

The diploid chromosome number in each sex is 14, confirming Nez's (J. Parasit. **40** [5, Sect. 2]: 37) counts in gametogenesis. The male complement is: 1 large V-shaped pair, about 4 microns long in sectioned material; 3 medium-sized V-shaped pairs of different sizes; two medium-sized J-shaped pairs, one with a satellite; and one small pair. The female complement is the same except that instead of the two largest V-shaped chromosomes there is one large V and a medium-sized rod-shaped chromosome. These largest V-shaped chromosomes, and the rod-shaped one in the female are interpreted as sex chromosomes and are designated as Z and W, respectively. The male, then, is AAZZ and the female is heterogametic with AAZW.

41. **Chromosomes in Parthenogenetic Miracidia of** *Schistosomatium douthitti* **(Tre-**

**matoda: Schistosomatidae) and their Progeny in Snails.** ROBERT B. SHORT AND MARGARET Y. MENZEL, Florida State University.

Chromosomes were studied (1) in parthenogenetic miracidial embryos within gut tissues of mice infected with female worms only, and (2) in cercarial embryos within laboratory-reared snails (*Lymnaea palustris*) infected with parthenogenetic miracidia obtained from mice and hamsters harboring female worms only.

Chromosome counts were made in 163 miracidial embryos from 4 mice. The haploid number (7) of chromosomes was found in 160 embryos and the diploid (14) in 3. Cells in an additional 5 embryos contained more than 7 chromosomes and were probably diploid although exact counts could not be made. All haploid cells contained one Z chromosome and all diploid cells two Z's.

Chromosomes were analyzed in 16 snail infections produced by parthenogenetic miracidia. Nine infections were male and 7 female. Five of the male infections were diploid; 3 were haploid; and one infection had both haploid and diploid cells. All haploid cells had one Z chromosome and all diploid cells 2 Z's.

Six female infections were diploid. The seventh had 15 chromosomes; 3 of the smallest chromosomes were present instead of 2 as normally found. All cells analyzed in these female infections were of the ZW type.

Haploid individuals of the chromosome constitution AZ develop at least to large cercarial embryos. Apparently AW ova do not survive. The diploid parthenogenetic males (AAZZ) are thought to result from doubling of the chromosome number of AZ egg cells or miracidial embryos, and the diploid parthenogenetic females (AAZW) probably result from unreduced eggs.

42. **Studies on Immunity to *Fasciola hepatica* in Rabbits.** GEORGE R. HEALY, The Rice Institute.

In rabbits previously immunized with extracts of *Fasciola hepatica* worm tissue or with pooled regurgitated cecal contents of worms, no evidence of resistance to infection was shown when the rabbits and controls were challenged with 50 metacercariae of *F. hepatica* and autopsied after 60 days. Some worms from the immunized group produced small numbers of abnormal eggs, while none of the control group did so. Such abnormal eggs have also been found occasionally in old infections of 7 months or more.

The prepatent period in *Fasciola*-infected rabbits was found to be 50 days although many worms do not have eggs *in utero* until 70 days.

As many worms develop in challenge infections in rabbits infected with *F. hepatica* for at least 60 days as in controls. When the animals are autopsied 35 days after the challenge infection there is no apparent effect on worms of the primary infection but there is some evidence that a retardation of development of challenge worms may occur, especially at periods earlier than 35 days after challenge infection, before the worms enter the bile ducts.

In rabbits infected with *F. hepatica* for periods of 30 days to 6 months, skin tests made with antigens prepared from extracts of *F. hepatica* and *Fascioloides magna* gave equally positive results when read at the end of 3 hours but tests made with *Schistosoma mansoni* were as negative as controls when read after the same period.

43. **Affinities of the Trematode Family Didymozoidae.** R. M. CABLE, Purdue University.

Fuhrmann and Poche have postulated that the DIDYMOZOIDAE may have been derived from the HEMIURIDAE and indeed many features of adult morphology and host relationship support that view. Further evidence is afforded by a study of the excretory system of immature trematodes common in the intestine of marine fishes and variously known as *Distomum fenestratum* and species of *"Torticaecum"* and *"Monilicaecum."* Manter's opinion that these forms are immature didymozoids is supported by finding a configuration of the anterior excretory tubules such as has been described for adult didymozoids and, furthermore, the system as a whole is very similar to that of certain hemiurids. It has been suggested that certain marine cystophorous cercariae with simplified tails and bodies remarkably like the immature forms mentioned are larvae of the DIDYMOZOIDAE. Experimental verification of that possibility would indicate clearly that the DIDYMOZOIDAE constitute a specialized group derived from hemiurid stock. An unspecialized marine cystophorous cercaria found in Puerto Rico provides an understanding of how the complex hemiurid cercaria may have been derived from a larva of the usual type and also indicates that the hemiurids belong to the PROSOSTOMATA and may be as closely related to the opisthorchioids as to any other group in that order.

44. **Studies on the Germ Cell Cycle of an Ochetosomatid Trematode. I. Gametogenesis in** *Neorenifer.* MARY C. DUNN, University of Georgia.

Development of male and female gametes has been studied cytologically in *Neorenifer.* The diploid chromosome number is 22, and 11 chromosomes appear in both spermatozoa and ova. In spermatogenesis the cells remain joined, finally forming a rosette of 32 spermatids. The nuclei elongate to form long thin spermatozoa which do not possess flagella. Oögonia are found near the periphery while oöcytes are found toward the center of the ovary. It appears that primary oöcytes are released from the ovary with a diffuse, post-synaptic nucleus, and that penetration by the spermatozoan is necessary for final maturation.

45. **Preliminary Studies on Hemagglutination in Schistosomiasis.** IRVING G. KAGAN, The University of Pennsylvania; AND JOSÉ OLIVER-GONZÁLEZ, The University of Puerto Rico.

Hemagglutination studies with immune rabbits and infected human sera were made. Seven rabbits were immunized with six injections of 2 mgs. of lyopholized egg, cercariae and adult antigens of *S. mansoni* suspended in Freund's adjuvant. Ten human sera before and one year after cure and 5 children sera with active infections of *S. mansoni were* tested.

Boyden's hemagglutination technique was used. The following lyopholized antigens (1:5000 in buffered saline pH 6.4) were employed as test antigens: cercariae, adults, eggs of *S. mansoni;* eggs of *S. haematobium;* eggs of *S. douthitti* in mouse spleens; cercarial and adult metabolic products (*S. mansoni*); human and pig ascaris; *Fasciola hepatica.*

Stage or species specificity was not observed with immune rabbit and human sera with the various antigens. Titers with cercarial antigen averaged 1:320 with anti-egg sera; 1:320–1:1280 with anti-cercarial sera and 1:1280 with anti-adult sera. The adult worm antigen was with one exception inactive, and the egg antigens yielded titers of 1:40–1:320. Cross reaction was noted with *F. hepatica* (1:10–1:20), but not with *Ascaris.*

Titers with children sera of 1:80–1:1280 with cercarial antigen, 0–1:320 with adult antigens and 1:320–1:1280 with egg antigens were obtained. Two sera cross-reacted with *F. hepatica* (1:10–1:20).

With 10 sera before treatment titers of 1:40–1:160 were obtained with cercariae and cercarial metabolic products antigen and 1:10–1:80 after treatment. Tests with egg antigens and adult metabolic products antigen were negative.

Titers of duplicate runs varied considerably. Studies are in progress to stabilize the test and to increase the sensitivity with human sera by utilizing purified antigens.

46. **Use of Organ Egg Counts in Assaying Chemotherapeutic Activity against** *Schistosoma mansoni.* THOMAS M. SCHWINK, Tropical Research Medical Laboratory, APO 851, New York, N. Y.

The methods used in drug screening in schistosomiasis have included (1) fecal examination; (2) worm counts; (3) observation of migration from intestinal veins to liver; (4) observation of survival time, presence of dead worms, and liver pathology; and (5) metabolic studies. The difficulties encountered in their use have tended to discourage large scale testing programs. In an attempt to find simpler quantitative methods an investigation of egg counts from tissues has been made.

Female white mice were exposed to 100 *S. mansoni* cercariae each by tail immersion. Graded doses of Fuadin were administered beginning five weeks after infection and the mice were sacrificed three weeks after the beginning of treatment (Luttermoser, 1954, J. Parasit. **40**: 130–137). To reduce the amount of feces in their intestines, the mice were starved overnight prior to being sacrificed. Livers and intestines of treated and control mice were placed intact in 5% NaOH solution and digested for 6 to 8 hours at 55–56° C. Egg counts were made on the residues. These showed an inverse correlation between the number of eggs and the size of the dose. Intestines proved more satisfactory than livers, and it is recommended that intestines alone be used in routine screening.

Advantages of this method include the following: (1) it is more rapid than many of the other techniques; (2) large numbers of animals can be killed on the same day and their organs refrigerated until time is available for processing and making counts; and (3) fewer cercariae are required than for survival time studies.

47. **Field-Test of Four Molluscacides against** *Oncomelania nosophora* **in the Terraced-Hillside Habitat.** ARTHUR P. MOON, YURI OTORI, JAMES E. WILLIAMS, LYMAN P. FRICK AND LAWRENCE S. RITCHIE, 406th Medical General Laboratory, APO 500, San Francisco, California.

In the spring of 1953, 1954, and 1955 four molluscacides were tested against *Oncomelania*

*nosophora* snails in an irrigated terraced-hillside habitat. A total of seven to eight miles of terrace was treated each time. The chemicals tested and quantities of active ingredient applied (grams per square foot) were: sodium pentachlorophenate (Santobrite), 0.4 or 0.8; dinitro-o-cyclohexylphenol (DN-1), 0.1 or 0.2; dinitro-o-secbutylphenol (Dupont Weedkiller), 0.1 or 0.2; and sodium 2,4,5-trichlorophenate (Dowcide B), 0.2 or 0.4. Application the first year was made with sprinkler cans; in 1954 these were replaced in part by gravity pressure spraying. In the third year a hand-operated pressure pump was used. Snail populations in all cases were based on the numbers of snails collected per man-hour. Santobrite treatment elicited mortalities of 95, 95 and 71 percent in the three applications compared to 84, 74 and 81 percent for DN-1. Cumulative population reduction for the period was 94 percent for Santobrite and 98 percent for DN-1. For Weedkiller and Dowcide B the figures were 90 and 65 percent. Less propagation of the snail appears to occur in the DN-1 treated area. In 1953 repopulation in the DN-1 area was 11 percent of the original population, whereas it was 47 percent with Santobrite. Corresponding figures in 1954 were 7 and 33 percent. 1955 figures are not yet available. While it has not actually been established that DN-1 affects the reproductive capacity of the snail, the possibility should be considered in the evaluation of molluscacides and emphasizes the desirability of parallel testing of molluscacides.

48. **Applications of Molluscacides against Juvenile** *Oncomelania nosophora* **for Repopulation Control.** JAMES E. WILLIAMS, ARTHUR P. MOON, YURI OTORI, LYMAN P. FRICK AND LAWRENCE S. RITCHIE, 406th Medical General Laboratory, APO 500, San Francisco, California.

*Oncomelania nosophora* exhibits a remarkable capacity to repopulate even when reduced to a population residue of essentially isolated snails. This seemingly is explained by the observation that females can propagate for two years after isolation. In 1953 this laboratory entertained the possibility of overcoming repopulation by applying molluscacides against the aquatic juvenile snails through the medium of the irrigation water. During the reproductive periods of 1954 and 1955 this idea was implemented. In test area I the adult population had previously been reduced to a low level, while in area II a very dense population existed. Dinitro-o-cyclohexylphenol (Dow's DN-1 formulation) was applied to the headwaters of each area three times at three-week intervals beginning about mid-July. Six-hour applications with 1 ppm (1954) and 2 ppm (1955) active ingredients were made and exposure further prolonged by stopping irrigation flow for at least 18 hours.

In 1954 repopulation in area I was very low, estimated at 0.2 offspring per original female. In 1955 the adult population was markedly reduced, and among 17 snails collected in about four man-hours only one was immature. Marked suppression of juvenile snails was evident in area II in 1954 at collecting sites exposed to direct flow of treated water. Results were inadequate only in ditches where unavoidable dilution occurred and where water-flow terminated after traversing rice fields. The adult population was little affected. An application of 5 ppm, designed to kill adults, was made in October 1954. Immediate benefits were negligible; however, appreciable mortality was evident the following spring. The 1955 2 ppm applications appeared to be highly effective in suppressing propagation; even destroying snails in the 3-5 mm. size range. Cumulative population reduction approaches 90 percent. A large scale trial appears warranted.

49. **A Restudy of the Genus** *Allassogonoporus* **(Oliver, 1938) Macy, 1940.** J. H. GILFORD, The Johns Hopkins University.

The genus *Allassogonoporus* was established by Oliver (1938) for *A. marginalis* from a muskrat, *Ondatra zibethica* L. Macy (1940) described *A. vespertilionis* from a bat, *Myotis californicus caurinus* Miller.

The cuticle of *A. marginalis* originally was reported to be without spines, although Oliver (personal communication) later was able to find cuticular spines in sectioned material. The acetabulum and genital pore are equatorial or post-equatorial and the vitellaria extend from the ovary to half-way between the intestinal fork and pharynx. Macy separated *A. vespertilionis* from *A. marginalis* by the presence in the former of a spined cuticle, the pre-equatorial position of genital pore and acetabulum, and the location of the vitellaria between the ovary and intestinal fork.

300 specimens of a species of *Allassogonoporus*, ranging from very young forms to mature adults, recovered from *Myotis lucifugus lucifugus* (Le Conte) and *M. sodalis* Miller and Allen have been examined. The relative positions of internal structures of the worm change during growth. Flattening during preparation for staining also will alter size, shape, and position of these structures. Morphological characteristics of type material of

*A. vespertilionis* and *A. marginalis* lie within the range of variability observed in the living and fixed material studied.

The genus *Myotitrema* Macy, 1940, contains a single species, *M. asymmetrica*, described from two specimens recovered from *M. lucifugus*. The type specimen appears to have been flattened considerably during fixation. It is concluded that morphologically *M. asymmetrica* is within the range of variability of *Allassogonoporus marginalis*.

The genus *Allassogonoporus* seems to be represented by a single known species, *A. marginalis* Oliver, 1838. *Myotitrema asymmetrica* Macy, 1940 and *Allassogonoporus vespertilionis* Macy, 1940 are considered synonyms of *Allassogonoporus marginalis*.

50. **Studies to Maintain Excysted Metacercariae of *Paragonimus westermani* in Vitro.** MUNEO YOKOGAWA, TOMOO OSHIMA AND MICHIE KIHATA, Institute of Public Health, Japan.

Three excysted metacercariae of *Paragonimus westermani* have been maintained in vitro for 98 days thus far (as of 2 Sept. 1955). From an average length of about 0.6 mm. and width of 0.26 mm. at rest immediately after excystation, these forms have grown to 2.0–2.5 mm. in length and 1.0 to 1.5 mm. in width at rest; stretched, they reach up to 4.0 mm. long. Signs of beginning development of the male reproductive system, especially of the testes, are being observed.

135 excysted metacercariae were used to start the cultures. Carrel flasks closed by rubber caps were employed, the cultures incubated at 37° C. and kept as sterile as possible including the use of antibiotics. The medium used for the first three weeks after excystation consisted of equal parts of cat serum and Tyrode solution. Chick Embryo Extract and cat blood cells were added to the medium of one third of the cultures and cat blood cells only was added to another third of the cultures after that time. All of the larvae in the media without both blood cells and Chick Embryo Extract died within eight weeks; all the larvae in the media with blood cells only died within twelve weeks. None of the worms in the media with both cat blood cells and Chick Embryo Extract died. The red blood cells and Chick Embryo Extract seem to be the important requirements for maintaining the larvae; it was observed that red blood cells were ingested.

It is felt that many more than three worms currently surviving would have lived up to this time; however, three to five worms were fixed and stained with hematoxylin for checking the development of the internal organs every ten days; thus greatly reducing the number of survivors.

The survival period of the larvae in cultures using serum from cats infected with *P. westermani* did not differ significantly from the survival period of those cultured in normal cat serum. A study to compare the survival time of the larvae in cultures using sera from various animals is now being undertaken.

51. **Respiration of *Paragonimus westermani*.** CLARK P. READ AND MARIANO YOGORE, The Johns Hopkins University.

*Paragonimus westermani* was obtained from the lungs of experimentally infected cats. In three experiments worms incubated in Krebs-Ringer phosphate (pH 7.2) containing 0.01 M glucose respired with $Q_{O_2}$ (μl. $O_2$/hr./mg. dry wt.) of 0.74 to 0.86. Animals exposed to a 30 minute period of anaerobiasis showed an oxygen debt when returned to air. The $Q_{O_2}$ in the first post-anaerobic 30 minutes varied from 1.72 to 5.06 and in the second post-anaerobic 30 minutes from 1.58 to 2.74. In all cases there was "over-payment" of the oxygen debt. These data may be compared with Bueding's findings that *Schistosoma mansoni* shows a higher $Q_{O_2}$ but does not contract an oxygen debt.

52. **Notes on the anatomy of *Ligula intestinalis* (Linnaeus, 1758), a Pseudophyllidean Cestode of Birds.** ELON E. BYRD, University of Georgia.

A single, sexually mature specimen (35.67 mm. in length) of *Ligula intestinalis* was made available for study in a small collection of worm parasites from the Grebe, *Colymbus auritus*, from Colorado. 137 sets of genital organs could be counted within the specimen and the first obvious set (whole mount) appeared 4.92 mm. behind the head-piece. The genital pores (uterine pore, vaginal opening and opening of the cirrus) are ventral in position: they irregularly alternate from side to side of the midline and lie in the bottom of a shallow, short, transverse genital depression. The vaginal opening always lies between the other two openings, and the successive groups of openings are separated by an average distance of 0.27 mm. The longitudinally bilobed ovary is always on the opposite side of the midline from the cirrus pouch. The testes are contiguous throughout the medullary parenchyma and number from 10 to 22 in a transverse section (in a sectioned piece 1.54 mm. long a total of 218 testicular

follicles was counted on one side of the midline, lateral to the genital organs). The testes are more numerous lateral to the nerve cord and are dorsal to that cord. In one series of sections a small ovarian mass was found directly ventral to one of the nerve cords. The vitelline follicles are external to the diagonal muscle layer and are contiguous except for a small area directly dorsal and ventral to the genital organs. A total of 10 excretory ducts was observed in each lateral half of the worm. Two of these ducts, the largest ones, lie in the medullary parenchyma, median to the nerve cord, while the other 8 on a side are located just outside the band of the vitellaria, one directly lateral, 2 ventral and 5 dorsal. In several instances short, lateral ducts could be seen passing into the parenchyma from the larger medullary tubules and in one instance a small duct was seen to connect this pair of tubules.

53. **Studies on *Echinococcus sibiricensis* (Rausch and Schiller, 1954) in Laboratory Mice.** SAROJAM K. MANKAU, Zoology Department, University of Illinois.

White mice are susceptible to *E. sibiricensis*. Numerous observations have been made on the establishment of the parasite in this host. Foci of infections (approximately 0.5 mm.) are evident in the liver about six days after infection; heavy leucocytic and connective tissue infiltrations, certain retrograde changes (glycogen infiltration, hyaline degeneration, cloudy swelling, hematogenous pigment deposition, etc.) and atrophy and necrosis of hepatic cells are characteristics of early development. The multilocular cystic growth-rate in white mice, which is by exogenous and endogenous branching, is comparatively much slower than in *Microtus* spp. In about 10 days, the germinal membrane is quite distinct and begins to proliferate; however, scolex-production does not occur until about the fourth month. Some white mice produce sterile cysts, while others produce both sterile and viable cysts. Viscera other than the liver often are found to be sites of infection. The fact that the animal remains fairly active in some cases even when 90% of the liver is cystic may be due to the compensatory action of the normal hepatic cells, which undergo extreme hypertrophy.

54. **Echinococcosis on St. Lawrence Island, Alaska.** LYELL J. THOMAS, Zoology Department, University of Illinois, and the U. S. A. F. Arctic Aeromedical Laboratory.

Continuation studies on *Echinococcus sibiricensis* Rausch and Schiller (1954) were made the summer of 1955 at North East Cape, on St. Lawrence Island, Bering Sea, Alaska. Rodents were trapped both within and outside of fox den areas northwest and southeast of the military installations. Ten of the total number of 74 *Microtus oeconomous* Pallas harbored the parasite cysts. This is a 13+% infection compared with a 16+% infection obtained last year. None of the 4 *Citellus undulatus* (Pallas) were infected. All six cub arctic foxes *Alopex lagopus* (Linnaeus) trapped had heavy infections of *Echinococcus* in addition to other tapeworms and nematodes. One old male fox taken was negative for *Echinococcus*. Of 20 adult foxes taken last April, only 3 had a light infection of this tapeworm. Studies on the viability of eggs after 8 months in water at 2° C. show that they are still viable. Other degrees of temperature and moisture effects on the eggs are being used. Immunity studies are now in progress with hydatid cyst antigen. An extensive study of alveolar cyst formation in white mice will soon be published.

55. **Immunity to *Hydatigera taeniaeformis* Affected by Radiation of the Host.** HONORICO CIORDIA AND ARTHUR W. JONES, The University of Tennessee.

Rats in which immunity to infection with the strobilocercus of *Hydatigera taeniaeformis* had been built up were subjected to 1250 roentgens of gamma radiation from a Co60 source. Subsequently they could be infected with larval *Hydatigera*. The host reaction interfered with seems to be part of the calcification process that destroys larval cestodes in immune hosts.

56. **The *in Vitro* Fermentation of Carbohydrate Substrates by *Hymenolepis diminuta* and the Action of Some Inhibitors.** J. S. LAURIE, The John Hopkins University.

A study has been made of the ability of *Hymenolepis diminuta* to utilize various carbohydrates which might be available in the gut of the host. The rate of evolution of carbon dioxide from Krebs-Ringer bicarbonate (pH 7.2 or 7.4 under an atmosphere of 95% $N_2$-5% $CO_2$ was used as a criterion of substrate utilization at 38° C.

D(+)glucose is rapidly metabolized, D(+)galactose somewhat less, and D(+)mannose still less. The corresponding reduction products D-sorbitol, D-dulcitol, and D-mannitol are not utilized. D(+)xylose is used to a slight extent. Sucrose, D(+)lactose, D(+)trehalose, D(+)maltose, D(+)glucosamine, glucosyloxime, α-methyl-D-glucoside, L(−)arabinose, i-inositol, salicin [o-(hydroxymethyl)phenyl-β-D-glucoside], mucic acid, glycerophosphoric acid, fructose-1,6-diphosphoric acid, and glucuronic acid fail to enhance acidic fermentation.

Salicin, lactose, and glucantime (N-methylglucamine antimonate) does not interfere with glucose utilization nor does lactose interfere in galactose utilization. Phlorizin [4,6-dihydroxy-2(β-D-glucosido)-β-(ρ-hydroxyphenyl)propiophenone] inhibits the utilization of exogenous glucose, galactose, mannose, and xylose but does not interfere with endogenous fermentation. The inhibition is readily reversed by a brief washing, indicating that inhibition occurs at or in the surface of the worm. The ready reversibility and the information that methylation of the phenolic hydroxyl groups or exposure to pH 8.3 renders phlorizin inactive suggests that "hydrogen bridges" may be important in the formation of the enzyme-inhibitor complex.

Quinacrine (atabrine) inhibits the anaerobic utilization of glucose. After addition, about 30 minutes are required for maximum inhibiton to be attained, suggesting that the site(s) of inhibition is intracellular.

When the data from kinetic studies of the glucose-phlorizin-cestode and the glucose-quinacrine-cestode systems are analyzed by the method of Lineweaver and Burk, both systems show the characteristics of non-competitive inhibition.

57. **Studies on Lipid Metabolism in Cestodes.** M. Warren and J. W. Daugherty, The Rice Institute, Houston, Texas.

Studies have been initiated on the nature and distribution of ether soluble material in the rat tapeworm, *Hymenolepis diminuta*. Approaches to the study of fat metabolism have been made by (1) host comparison studies using Sprague-Dawley and Wistar rats and golden hamsters as hosts, (2) determination of the linear distribution of ether soluble material in the tapeworms, and (3) studies on the effect of variations in the lipid content of the host diet on the parasite. Analytical methods have, to the present time, included the measurement of total lipid content of the parasite tissues and an estimation of the degree of saturation of the fatty acids involved. The investigation has disclosed that both the type of host and its diet are effective in determining the total fat content of the parasite and the nature of the lipid material present.

58. **The Effect of Fasting and Alloxan Treatment of the Host on Glycogen Gradient in *Hymenolepis diminuta*.** Jack W. Daugherty, The Rice Institute.

At the present time there is little information concerning variations in the physiology and biochemistry relative to the different anatomical regions of tapeworms, although a few preliminary quantitative observations have been made. In consideration of the marked regional differences in the anatomy of tapeworms, especially with reference to the maturation of the reproductive organs and the development of eggs and embryos, it is possible that there may exist quantitative differences in intermediary metabolism.

Mature *Hymenolepis diminuta*, raised in male white rats of the Sprague-Dawley strain, were used throughout. In the control worm the glycogen content is high in the region nearest the scolex, but drops in the next ten centimeters, rises in the succeeding ten to twenty centimeters, plateaus briefly, and, finally, progressively declines to the last of the gravid proglottids. A parallel study was made on the glycogen content of mature tapeworms taken from infected hamsters. Fasting the rat host for twenty-four hours had a pronounced effect on the glycogen content of the first two or three 10 cm. sections of the worm but little effect on the posterior 50 cm. Following alloxan treatment of the host the major effect was in the anterior portion of the worm, where, in the first 10 cm. section, the glycogen level was raised from a fasting low of *ca.* 7 mgm./gm. to over 75 mgm./gm. In the more posterior sections the elevation of the glycogen content was progressively less.

59. **A Comparison of the Amino Acid Constituents and the Transaminase Activity of *Raillietina cesticillus* and *Hymenolepis diminuta*.** William B. Foster, The Rice Institute.

This study is the beginning of a comparison of the protein metabolism of these two tapeworms. An analysis of extracts of *Raillietina* by paper chromatography has shown the following free amino acids and related substances to be present: analine, aspartic acid, citrulline and/or glutamine, glutamic acid, glutathione, glycine, proline, serine, tyrosine, and valine. Probably leucine, isoleucine, and methionine are also present. Arginine, cystine, lysine, and taurine, identifiable in *Hymenolepis*, are not found in *Raillietina*. However, proline is present in a much greater quantity in the latter worm.

Of the four transaminase systems that previously have been found active in *Hymenolepis*, one, the glutamic acid to alanine system, could not be demonstrated in preliminary studies on *Raillietina*, and the other three systems appear to differ quantitatively between the two worms.

60. **The Relation Between Lesions in Lambs and the Development of the Cysticerci**

**of *Taenia hydatigena* Pallas, 1766.** GORDON K. SWEATMAN, Animal Pathology Division, Canada Department of Agriculture, Branch Laboratory, McGill University, Macdonald College, Quebec, in cooperation with the Institute of Parasitology.

Lesions and other damage caused by developing cysticerci of *Taenia hydatigena* in lambs were checked against the period of infection. Lambs killed seven days following exposure to 800 ova showed small fibrotic lesions on the surface of the livers. Between 10 and 25 days after exposure haemorrhagic streaks were also present, each of which contained one or at most two cysts that measured about two mm. at 12 days and up to nine mm. at 20 to 25 days. All streaks were repaired after 25 days, but some of these and other caseous lesions in the liver that persisted for varying periods up to nine months contained cysts that never reached a size greater than nine mm. and which remained barren of hooks and suckers. Open pits in the omentum and liver capsule first appeared in a 20 day infection and it was at this time that the greatest percentage of cysts occurred unattached in the abdominal cavity. Such cysts were last observed 23 days later. Some cysts located within the abdominal cavity or attached to the omentum, mesenteries or the ligament joining the bile duct and liver measured ten mm. or more in a 34 day infection and were the first cysts observed that possessed hooks and suckers. Cysts not only damaged the liver and omentum but also caused local peritonitis and the formation of various adhesions and ribbons of fibrotic tissue, most of which involved the liver and diaphragm or the omentum and body wall.

61. **Glycogen Synthesis in *Hymenolepis diminuta*.** JACK W. DAUGHERTY, The Rice Institute.

A description of the process by which glycogen is synthesized from the simple sugars available in the intestine of the host and a knowledge of the controls pertaining to this function are essential to an understanding of the physiology and host-parasite relationship of *Hymenolepis diminuta*.

Mature worms were washed in several changes of Krebs-Ringer-phosphate, blotted, weighed, and *ca.* 250 mg. portions of the anterior ends of separate worms were placed in chilled flasks containing 5 ml. of the following incubation medium: $CaCl_2$ (0.01M), KCl (0.65M), K-Pyruvate (0.04M), or Glucose (0.02M), $KHCO_3$ (0.008M), and $NaHCO_3$ (0.03M).

Both the glucose and pyruvate fortified media supported glycogenesis. Castration of the rat host produced conditions that caused a decline in the rate of glycogen synthesis from both glucose (difference of castrate and normal means = $23.1 \pm 8.1$) and pyruvate (difference of means = $29.7 \pm 8.4$). There were some indications that fasting the rat host for 24 hours prior to the experiment permitted a higher rate of glycogen synthesis from glucose. It is apparent that, as had been pointed out elsewhere, the rat tapeworm has not entirely lost the ancestral ability to regulate its internal physiological processes in response to environmental conditions. In addition, it seems probable that the broad effects of castration on the physiology of the rat tapeworm are produced through a secondary set of conditions arising in the body of the host and should not be attributed to any direct effect of the absence of the hormone on the parasite.

62. **Experimental Demonstration of the Life Cycle of *Hymenolepis citelli* (McLeod, 1933) (Cestoda: Cyclophyllidea).** M. VOGE, University of California, Los Angeles.

Infective cysticercoids of *Hymenolepis citelli* were obtained from laboratory reared beetles (*Tribolium confusum*) 14 days after feeding them eggs from an infected groundsquirrel (*Citellus beecheyi*). Five of these cysticercoids were fed to an uninfected groundsquirrel of the same species kept in the laboratory for one year prior to the experiment. Eggs were passed by this groundsquirrel 18 days after infection. Autopsy revealed five fully mature *H. citelli*. A laboratory rat (Long-Evans) infected the same day as the groundsquirrel harbored 3 *H. citelli* after 18 days, but the worms, although not abnormal, were not fully mature and were less than one half as long as those from the groundsquirrel.

Comparison of cysticercoids of *H. citelli* and *H. diminuta* grown in *Tribolium confusum* for the same number of days showed distinct structural differences as well as consistent discrepancies in size relationships.

63. **Studies in Embryology and Histology of the Robin Tapeworm, *Choanotaenia iola* Lincicome (Cyclophyllidea: Dipylidiinae), with Description of the Metachromatic Glands in the Onchosphere.** R. E. OGREN, Biology Department, Ursinus College, Collegeville, Pa.

Oocytes contained large chunks of vitelline substance. Oocytes underwent meiosis in the uterus. Zygote divided into two nearly equal blastomeres. One of these failed to take part in development and was excluded from the embryo. The other divided into numerous blastomeres cleaving at different rates to form the morula. The large onchosphere was enclosed

by the shell capsule and the pseudoembryophore, a region of membranes and granules with a metachromatic reaction. The body of the onchosphere was composed of somatic cells forming the parenchmya. Squamous epidermal cells at the embryonic posterior, failed to detach from the inner cell mass to form an embryophore. These granule filled cells developed during hook formation and later became U-shaped glands; Reid's penetration glands. The granules were metachromatic and stained purple with toluidine blue, red with safranine O, yellow with neutral red *in vivo*, red with the Periodic Acid Schiff reaction, red with eosin. The granules were involved in formation of membranes of the pseudoembryophore.

64. **The "Nasal-Leech" *Dinobdella ferox* from Borneo and Malaya.** Bryce C. Walton, Army Medical Service Graduate School.

*Dinobdella ferox* (Blanchard) is a leech which commonly invades the throat and nasal passages of man and animals, where it remains for considerable periods of time. There are few reports in the literature concerning this leech in spite of its attacks on man, the serious effects it may cause, and a widespread distribution in the orient. It has been reported as occurring in India, Burma, Formosa and southeastern China (Moore 1949, quoted by Chin, J. Parasit. **35:** 215) and attacking man, dogs, horses, cattle, deer, monkeys and "other wild animals." A joint U. S. Army-British Colonial Office Field Team investigating scrub typhus and animal reservoirs of disease made collections which constitute new locality records and a new host record. Engorged specimens were taken from the nasal passages of the large water rat (*Rattus mülleri*) at Ulu Langgat, Malaya, and from the noses of dogs at two localities on Mt. Kinabalu, North Borneo. Immature specimens were also found in great numbers in streams in remote areas seldom visited by man or domestic animals, indicating that it does not live solely in the vicinity of human habitations. These were established in laboratory rats and very rapid initial growth was observed. All specimens obtained from hosts in Borneo and Malaya showed only rudimentary reproductive systems. This agrees with observations made by Moore (1927, Fauna of British India), that the reproductive system develops only in free-living individuals and not in those that become parasitic.

65. **Factors Influencing the Susceptibility of Mosquitoes to Malarial Parasites.** Don W. Micks, The University of Texas Medical Branch at Galveston.

The effects of various vitamins, amino acids and blood fractions upon the susceptibility of *Culex fatigans* to *Plasmodium cathemerium* were investigated. These factors were administered individually (*i.v.*) to infected canaries just after allowing control batches of mosquitoes to take blood meals and immediately before feeding the test groups. After 7–9 days, the stomachs of all specimens were dissected out. The percentage of infection and the average oocyst number in both the test and control groups were compared in order to detect any alteration in mosquito susceptibility to the parasite. The specific effects of various nutrients at different levels of concentration will be discussed. (This investigation was supported by research grant G-1978 (C3) from the National Microbiological Institute of the National Institutes of Health, Public Health Service.)

66. **Quantitative Microinjection of Mosquitoes.** J. Chao and G. H. Ball, University of California at Los Angeles.

In *in vitro* studies of oocyst development, oocysts of *Plasmodium relictum* free from the stomach wall of *Culex tarsalis* were sought by injecting a known quantity of infected canary blood into the haemocoel of the mosquito. The quantity of the blood injected could be calibrated accurately by using a glass capillary made according to the principle of the "braking" pipette. This was composed of a capillary inserted and sealed into glass tubing which was attached to a rubber bulb for injection. The capillary filled automatically when it touched the heparinized or citrated blood and stopped when full. The mosquito was held by suction to glass tubing, which was clamped to an apparatus providing three dimensional movement. In the injection carried out under the dissecting microscope, the mosquito was moved to the capillary which was kept stationary. The technique was checked by injecting heparinized or citrated saline solutions into the mosquitoes. The mortality was almost nil as compared with the controls but it increased drastically with the presence of blood. After two weeks, the surviving mosquitoes injected with infected blood were dissected and examined for oocysts and sporozoites. The results were negative. Mosquitoes fed artificially with a micropipette containing some of the same sample of blood employed for injections developed infections normally. (This investigation was supported in part by Grant 1857 from U. S. Public Health Service.)

67. **Sterile Microdissection and Isolation of Malarial Oocysts from the Mosquito Stomach.** J. Chao, University of California at Los Angeles.

Oocysts of *Plasmodium relictum* were dissected under sterile conditions from the stomach wall of infected *Culex tarsalis* for *in vitro* culture studies. The dissections were done in a chamber which was sterilized by spraying it with 70% alcohol. It was equipped with all necessary sterile articles including Ringer's solution, hexylresorcinol, culture medium, petrolatum-ringed depression slides, etc. The mosquito was sealed with Duco cement at both the oral and anal openings, sterilized by dipping it briefly into hexylresorcinol, rinsed through Ringer's solution, removed from the body wall under low power of the dissecting microscope, and dissected for oocysts under high power without cutting through the stomach wall. The oocycts were driven on to a small sliver of broken coverslip, which was picked up by fine forceps and placed into the medium for culturing. For a steady grasp of the dissecting needles, each member of the pair was fastened to the side-slit of the microscope-stage by a piece of thick soft wire sheathed in plastic. For better contrast of the object, two beams of light were focused through the Lucite top of the chamber, and a physicists' black-body was employed in the middle of the microscope-stage, that is, a simple hole was provided which prevented reflection on the object from below. A foot-pedaled focusing device was provided to free the hands for dissection. Oocysts of the size of 20 mu have been dissected and successively transferred by this technique. (This investigation was supported in part by Grant 1857 from U. S. Public Health Service.)

68. **The Tergal Plates of North American *Anopheles* Larvae—Their Use in Identification and Phylogenetic Studies (Diptera: Culicidae).** HARRY D. PRATT, Communicable Disease Center, U. S. Public Health Service, Atlanta, Ga.

The larvae of North American *Anopheles* may be divided into three groups according to the arrangement of tergal plates: Group I including *albimanus, franciscanus,* and *pseudopunctipennis* have tergal plates on the thorax and four tergal plates on each abdominal segment; Group II including *barberi, quadrimaculatus, atropos, walkeri, crucians, georgianus, bradleyi,* and *punctipennis* have no tergal plates on the thorax and only two tergal plates on each abdominal segment; Group III including *aztecus, earlei, freeborni,* and *occidentalis* have no tergal plates on the thorax and four tergal plates on each abdominal segment. Maps are presented showing the extension from Central America of Group I and the northern, possibly Holarctic, origin of Group III which might be called the American *maculipennis* group. The arrangement of tergal plates has a practical value also since the important vector of malaria in Western North America, *A. freeborni,* has four tergal plates on each abdominal segment while the closely related *A. punctipennis* has only two tergal plates.

69. **Studies on the Clinical Effects of the Screw-worm, *Callitroga hominovorax* (Coquerel) on Guinea Pigs.** JACK H. ESSLINGER, The Rice Institute.

Clinical studies were made on guinea pigs experimentally infested with light, heavy, and lethal numbers of screw-worms. Attention was given to general condition and behavior, weight, temperature, erythrocyte counts and leucocyte counts of the hosts.

About the third day after infestation heavily infested guinea pigs began to show a sharp drop in weight, a marked rise in temperature, and a leucopenia, all reaching an extreme between the fourth and sixth days. About the fifth day a drop in the erythrocyte count began and by the sixth day a definite anemia had developed. These manifestations coincided with the period of maximum growth of the third instar larvae. By the time the maggots had left the host (8th day) animals had begun to gain weight, and temperature and leucocyte counts were back to normal. The erythrocyte counts started returning to the normal range after the eighth day.

Since the symptoms reached their peak before tissue destruction and decomposition was at its height, a toxic action by the growing parasites is suggested.

In contrast to this, in infestations by the secondary screw-worm (*Callitroga macellaria*) no comparable toxic effects were observed.

70. **Experimental Studies on Argasid Ticks as Possible Vectors of Tularemia.** WILLY BURGDORFER, Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Microbiological Institute, Rocky Mountain Laboratory, Hamilton, Montana.

In connection with studies on ticks of the genus *Ornithodoros* as possible vectors of various disease agents, the species *Ornithodoros moubata, O. turicata, O. parkeri,* and *O. hermsi* have been tested for their ability to ingest, preserve and transmit *Pasteurella tularensis.* All ticks were infected by feeding on mice which had been previously inoculated with *P. tularensis.*

The development of the organisms in the ticks was followed for 450 days by means of microscopic examination of histological sections and smears. The observations made show

that the bacteria arrive with the infectious blood in the diverticula of the midgut where they invade the amoebic cells of the gut wall. The multiplication of the organisms in the cell cytoplasm produces distended cells which eventually rupture and discharge their contents into the body cavity. Following this, invasion of the salivary glands, central ganglion, coxal organs, and tissues of the genital and excretory systems takes place. The coxal fluids of *O. moubata*, *O. turicata*, and *O. parkeri*, as well as anal excretions of all four species, contained large masses of *P. tularensis*.

A total of 472 transmission experiments, in which the ticks were fed individually or in lots of 2 to 5 per mouse, was performed. All species tested were able to transmit *P. tularensis*, but the most efficient vector was *O. moubata*. The results suggest that argasid ticks may be of importance in the maintenance and distribution of *P. tularensis* among their natural hosts.

(Paper will be published in extensio in JOURNAL OF INFECTIOUS DISEASES, 1956.)

71. **Food Preference and Longevity of the Spiny Rat Mite,** *Echinolaelaps echidninus*. R. W. STRANDTMANN AND VIRGINIA LANDWER; Texas Technological College.

Utilizing the willingness of *Echinolaelaps echidninus* to feed on free blood droplets, groups of mature female mites were offered a choice of whole human blood, whole rat blood, whole sheep blood and water. Rat and human blood were about equal choices, with rat blood a slight favorite. Sheep blood was a poor third and water was completely ignored.

To test the effects of each of these bloods on longevity, three groups of 100 mature female mites, chosen at random and starved for 24 hours were allowed to feed once on one of the three bloods and then kept without further food at 80% relative humidity and 28° centigrade. Results are not yet available.

72. **Observations on the Life Cycle of the Parasitic Mite** *Chorioptes bovis* **(Gerlach) Using an** *In Vitro* **Technique.** GORDON K. SWEATMAN, Animal Pathology Division, Canada Department of Agriculture, Branch Laboratory, McGill University, Macdonald College, Quebec, in cooperation with the Institute of Parasitology.

A technique has been developed for the *in vitro* maintenance of *Chorioptes bovis* on a substrate under constant conditions of complete darkness, a temperature of 35° C. and a relative humidity of 80%. Some observations have been made on the life cycle of the mite using this method. Eggs hatched four days after being laid. The larvae became quiescent at various times between 7 and 11 days following the laying of eggs and remained in that state for approximately 24 hours. The nymphs that emerged became quiescent for a similar period as the larvae at various times between 12 and 17 days after oviposition. Following this ecdysis, adult males appeared which copulated immediately with pubescent females. Continuous copulation persisted for a few days, and then the females molted into ovigerous females and began to oviposit three to seven days later. Exceptions were noted, but the usual period for the complete life cycle was 22 to 27 days.

73. **"Parasitism and Parasitology".** CLAY G. HUFF, Naval Medical Research Institute, Bethesda 14, Maryland. **Presidential Address.**

74. **Development of Megaloschizonts of** *Leucocytozoon simondi*, **Mathis and Léger 1910, and Some Host Reactions.** ARCHIBALD B. COWAN, Patuxent Research Refuge, U.S. Fish and Wildlife Service, Laurel, Maryland.

Histological studies of megaloschizonts of *L. simondi* resulted in an interpretation of their mode of development which differs from that of Huff (1942). This concept is based upon examination of tissue sections taken from infected white Pekin ducklings during the first four days of parasitemia.

Megaloschizonts developed from trophozoites. The trophozoite nucleus became dense with chromatin and buds formed on the periphery. At this stage the nucleus began to resemble the central body of Huff (1942). The buds were set off into the cytoplasm to form cytomeres. In the cytomeres, chromatin increased and concentrated into peripheral clusters which separated to form new cytomeres. Production of cytomeres continued by both methods until the megaloschizont reached its maximum size and was completely filled with cytomeres. Subsequently the central body ceased to bud and division by the cytomeres produced progressively smaller, but more deeply staining, bodies. Ultimately, the central body was reduced to an amorphous mass; merozoites were formed; and the megaloschizont ruptured releasing the merozoites into the adjacent tissues.

It was also observed that host cells or their products can successfully attack and destroy

megaloschizonts. Heterophils, either alone or with the aid of lysins, penetrated megaloschizonts and started the destruction, probably by both phagocytosis and lytic action. Macrophages followed and appeared to either replace or displace the heterophils within the parasite and ultimately completed the removal. In some cases lysins alone attacked and destroyed megaloschizonts. Reticular cells appeared to be the prime defense cells in the brain. They first concentrated around the schizonts, often causing marked injury to adjacent brain cells in the process, and then invaded and destroyed the parasite.

Although this interpretation is based only on observations on fixed tissues, the graded series of stages observed in this material justifies presentation of the foregoing hypothesis. The cycle discussed above is considered to represent only the final generation of prepatent schizogony.

75. **Precipitates on the Larvae of *Litomosoides carinii* in Sera of Repeatedly Infected White Rats.** NORMAN T. BRIGGS, The University of Texas Medical Branch, Galveston, Texas.

The demonstration will include precipitates and photographs of precipitates on the infective larvae of *Litomosoides carinii* in sera of repeatedly infected white rats and cotton rats, as well as the absence of precipitates in sera of uninfected white rats and cotton rats. The partial natural immunity of similar uninfected white rats to this species of worms is so near absolute that it has been virtually impossible to demonstrate any acquired immunity over and above the natural immunity. It is interesting, then, that precipitates form in sera of repeatedly infected white rats and not in sera of uninfected white rats. It might be argued from these results that the precipitates have little to do with the natural immunity of the white rats.

76. **Some Microfilariae From Birds, With A Graphic Demonstration Of Species Differences.** EDWIN J. ROBINSON, JR., Kenyon College.

(No abstract submitted)

77. **Anomalies in *Fasciola hepatica*.** GEORGE R. HEALY, The Rice Institute.

Some 200 flukes obtained from cattle at Houston abattoirs were stained and mounted to compare with worms from experimentally infected rabbits. In both groups of animals 10% of the worms had the ovary sinistral in position. In addition to right or left position of ovaries, a few cattle flukes had ovaries in which the lobes occupied various positions. Some specimens had the ovarian lobules equally distributed on either side of the mid-line and two specimens were found in which the lobes were arranged to form a cross, ventral in position.

Among the cattle flukes a few specimens, 20–30 mm. in length, had pycnotic vitellaria. One specimen (23 mm. long × 5 mm. wide) from cattle did not possess vitellaria or testes.

78. **The Life-Cycle of *Azygia sebago* Ward, 1910.** HORACE W. STUNKARD, New York University.

Stunkard (1950) reported the asexual stages of a species of *Azygia* from the pectinibranchiate snail, *Amnicola limosa*, in the region of Woods Hole, Massachusetts. Adult stages of the parasite have been found in only one host species, *Anguilla rostrata*. Planarians and small fishes, which are eaten by eels, serve as paratenic hosts. The cercariae were snapped up and persisted for a long time in the stomachs of guppies and small blue-gill sunfish, *Lepomis macrochirus*. Comparison of the worms from the eel with published descriptions and with specimens from the U. S. National Museum indicates that they are identical with worms described by Ward (1910) from land-locked salmon and other fishes of Lake Sebago, Maine as *Azygia sebago*. Ward's description may have included material of more than one species. The three species described as new by Goldberger (1911): *Azygia acuminata*, *Azygia bulbosa*, and *Hassallius hassalli*, may be identical with *A. sebago*. *Azygia angusticauda* (Stafford, 1904) appears to be a valid species and *Azygia longa* (Leidy, 1851) may be distinct from *Azygia lucii* (Mueller, 1776), although both are from the same host species, *Esox lucius*.

79. **The Gland Complex of the Cercaria of *Schistosoma mansoni*.** F. J. KRUIDENIER, University of Illinois; AND M. A. STIREWALT, Naval Medical Research Institute.

Histochemical analyses were made of the cercaria of *Schistosoma mansoni* within infected snails (*Australorbis glabratus*), normally emerged, penetrating mouse skin, exposed to normal mouse serum and to the serum of schistosome-infected mice. Special emphasis was placed on secretory activities of the cercariae. Two pairs of pre-acetabular predominantly "eosinophilic" glands and three pairs of post-acetabular "basophilic" glands were differentiated.

Pre-acetabular glands are diffusely purpurin-positive (Alizarin 6) and demonstrate

irregularly scattered areas of Hotchkiss-positive, Bauer- and Casella-negative material. Their secretions are present in the glandular discharge of free-swimming cercariae and of active penetrants. Post-acetabular glands are strongly and generally Schiff-positive. Their discharge was traced to the fine extra-cuticular film that is closely applied to the general surfaces of immature cercariae within sporocysts and is retained through the early stages of normal penetration. The Schiff-positive film is the cercarial contribution to the pericercarial envelope (CHR) produced around cercariae exposed to the serum of schistosome-infected mice. The discharge of the post-acetabular glands continues through the free-swimming stage and massive emissions aid cercariae during skin penetration.

80. **The Nervous System of Some Digenea.** NEWTON KINGSTON AND DOMINIC L. DEGIUSTI, Wayne University, Department of Biology.

Presenting by demonstration the morphology of the nervous system of the family RENIFERINAE, and in part the nervous system of *Schistosoma mansoni* and *Spirorchis* sp.

81. **Morphology of the Adult and the Miracidium of a Progenetic Species of *Allocreadium* from Water Beetles of the Family** DYTISCIDAE. L. E. PETERS, JR., University of Michigan Biological Station and Purdue University.

A species of *Allocreadium* occurs unencysted in the body cavity of the dytiscid water beetles, *Dytiscus fasciventris* and *Acilius semisulcatus*, collected from small pools near Douglas Lake, and may be the same species as the one reported from *Dysticus* sp. in Colorado by Crawford (1940). The trematode not only is progenetic to the extent of attaining full maturity and laying large numbers of eggs but also is neotenous to the extent that a stylet is present and eyespots remain virtually intact. These larval characters imply that the cercaria is of the type known for the papillose allocreadiids and thus gives additional evidence of a close relationship between *Allocreadium* and *Crepidostomum*. The excretory system of both genera has the same number of flame cell groups and the pattern in the species from beetles is intermediate in complexity between that of *Crepidostomum* spp. and *Allocreadium ictaluri*. Furthermore the miracidium of the present species resembles that of *C. ictaluri* more than that of *A. ictaluri* in lacking a stylet and having an epidermal cell formula of 6-6-4-2.

An ophthalmoxiphidiocercaria having a stylet comparable to that of the adult from beetles was found to develop in *Sphaerium occidentala* in a temporary pool where beetles were infected but fish were absent. While a vertebrate may play the usual role of definitive host to this species, such a host obviously is not necessary for the completion of the life history.

82. **Encephalitis in a Swan Due to *Dendritobilharzia* sp.** NORMAN D. LEVINE, DAVID T. CLARK AND LYLE E. HANSON, University of Illinois.

*Dendritobilharzia* sp. was found in a small artery in the brain and in the mesenteric blood vessels of a two-year-old swan which had been raised in Illinois and which had died with symptoms of encephalitis. On histopathological examination, the cerebrum was found to be peppered with fluke eggs. Eggs were also found in the liver, spleen and villi of the small intestine.

83. *Nematobothrium texomensis* **n. sp. from a Freshwater Fish,** *Ictiobus bubalus* **(Rafinesque, 1819).** ALLEN MCINTOSH, Parasite Laboratory, Animal Disease and Parasite Research Branch, ARS, U.S.D.A. Research Center, Beltsville, Md.; AND J. TEAGUE SELF, Department of Zoology, The University of Oklahoma, Norman, Okla.

Two specimens of this parasite were found entangled among the ovaries of a small-mouth buffalo fish from Lake Texoma, Willis, Oklahoma, in April 1955. The collector of the material reported that the two very narrow, elongated specimens had a total length estimated in excess of 17 feet and could not be removed entire, but only in numerous short pieces. The species is believed to be new and belongs to the trematode family DIDYMOZOIDAE Poche, 1907. Considering its narrow, long, winding body this species is assigned to the genus *Nematobothrium* van Beneden, 1858.

*Description*: Estimated length between 8 and 9 feet, subcylindrical, diameter about 1 mm.; cuticle aspinose. Oral sucker subterminal, feebly muscular; pharynx muscular, 0.24 mm. long by 0.2 mm. wide; esophagus 0.65 mm. long by 0.03 mm. wide; ceca long. Acetabulum poorly developed, 2 mm. from anterior end, 0.09 mm. in diameter. Testes and ovary long, tubular, location in body undetermined. Vitellarium single, long, tubular, extending to posterior end. Uterus filling most of body not occupied by other organs, first ascending, then descending to posterior extremity, reversing, and ascending to genital pore near anterior extremity.

Eggs numerous, subglobular, 0.025–0.03 by 0.022–0.025 mm., shell thin, transparent. Type, U.S.N.M. Helm. Coll. No. 37476, from Lake Texoma, Oklahoma; paratype, 18852, from Atchafalaya River, Louisiana. This is the first record of any didymozoid trematode from an American freshwater fish It appears to differ from all other members of the genus in that the oral sucker is extremely small in relation to the strongly muscular pharynx.

84. **Transfaunation of Adult *Hydatigera taeniaeformis* to Its Intermediate Host.** ARTHUR W. JONES AND HONORICO CIORDIA, The University of Tennessee.

Whole strobilae and pieces of adult *Hydatigera taeniaeformis* were placed within the abdominal cavities of non-immune laboratory rats. After several weeks the cestodes were found in various locations within the host. Encapsulation with connective tissue and infiltration with leucocytes (chiefly heterophile) had proceeded, but the cestodes were still motile, and their tissues had not undergone marked deterioration. In each case, the scolex and neck regions were embedded in or upon the liver of the host.

85. **Acanthocephala of the Genus *Centrorhynchus* from Birds of Egypt.** HELEN L. WARD, The University of Tennessee, Knoxville.

Numerous specimens of the genus *Centrorhynchus* have been collected from birds in Egypt by parasitologists of the United States Naval Medical Research Unit No. 3. These specimens represent four different species, two of which have been found to be new. Perhaps describing the new species are in press. One new species was found from the kite, *Milvus migrans*, and another new species from the falcon, *Falco tinnunculus*. In addition, *Centrorhynchus corvi* Fukui, 1929, was found in the crow, *Corvus corone*, and *C. globocaudatus* (Zeder, 1800) was found in the owl, *Athene noctua*.

86. **Nymphal Stages of *Pneumonyssus simicola* (Acarina: Halarachnidae).** WILLIAM B. HULL, Naval Medical Research Institute.

The nymphal stages of the monkey lung mite, *Pneumonyssus simicola* Banks, have been seen by few, if any, workers in the past. This has caused several investigators to conclude that no nymphal stage exists. The author has a mounted nymphal stage (with molted larval skin on same slide), which is interpreted to be a protonymph containing both the deutonymphal and adult stages within. Two other mounts of larvae, each containing a (proto?) nymph are also on hand. Several figures to illustrate these specimens, plus appropriate figures of normal larvae and adults for comparison, will be displayed.

87. **Penetration of Host Tissue and Host Reaction to Chigger Mites of the Genus *Hannemania* (Acarina; Trombiculidae).** K. E. HYLAND, JR., University of Rhode Island; AND G. W. WHARTON, University of Maryland.

In contrast to the truly ectoparasitic habit common in trombiculid mite larvae, larvae in the genus *Hannemania* generally become embedded within the integument of the amphibians to which they attach. When a site for attachment has been determined the chelicerae are inserted into the epithelium and the mite enters at an angle of 30 degrees or more. It penetrates gradually into the integument and within several hours has progressed the entire length of the mite. As a result it is covered dorsally by host tissue a few layers in thickness and the orifice through which it entered lies posterior to the larva. This orifice closes and the mite is wholly within the integument. The dense connective tissue layer of the skin is not broken by the mite, but seems to serve as a barrier which prevents the larva from penetrating to deeper tissue.

Penetration appears to be primarily a mechanical phenomenon. No enzymes have been demonstrated nor have there been indications of enzymatic activity from a histologic study. Once the chigger becomes located within the skin the loose connective tissue becomes more extensive and thicker while the dense connective tissue beneath the mite becomes thinner. Later the connective tissue around the mite becomes continuous and forms a complete protective envelope or capsule which is sometimes broken in the area of the gnathosoma of the mite.

88. **An Apparatus for the Continuous Collection of Ectoparasites from Nests of Small Mammals.** ROGER O. DRUMMOND, Department of Zoology, University of Maryland.

Ectoparasites are easily obtained from small mammals by collecting them from the mammals themselves and from their nests. The ectoparasites are usually removed from the nests by a Berlese apparatus. This apparatus employs heat and light to drive the organisms from the nest. Since the Berlese method destroys the nest, only a single collection can be

made from a nest. A new apparatus was constructed in order to obtain a continuous record of the populations of ectoparasites in the nest.

This new apparatus consists of a combination of the funnel from the Berlese apparatus with the nest box which is used in the study of populations of small mammals. The nest boxes are modified by putting in a bottom of fine mesh hardware cloth. A funnel is attached to each box. The funnels have bottle caps soldered to the narrow end so that bottles may be exchanged without removing the funnels from the boxes. Bottles are collected weekly and the number of ectoparasites is recorded.

Boxes, used in studying the nest inhabiting ectoparasites of *Peromyscus leucopus noveboracensis* (Fischer), have the dimensions of a six-inch cube. The bottom of the boxes is ¼" hardware cloth. Examples of weekly collections are given. (This investigation was supported by the Medical Research and Development Board, Office of the Surgeon General, Department of the Army, under Contract No. DA-49-007-MD-436 and was done at Patuxent Research Refuge, Bowie, Maryland, with the cooperation of the U. S. Fish and Wildlife Service.)

89. **"Schistosomiasis in Egypt."** ROBERT E. KUNTZ, Naval Medical School, NNMC, Bethesda, Maryland.

This film is a pictorial report on schistosomiasis as seen in Egypt. It touches briefly upon the history, distribution, treatment, epidemiology, etc. of schistosomiasis in Lower Egypt and illustrates some of the methods employed by the government in attempting to bring this debilitating disease under control. Some consideration is given also to the activities of other organizations such as Naval Medical Research Unit No. 3 taking part in the study of schistosomiasis.

90. **A Motor-Driven Enterotome for the Rapid Removal of Helminths from the Gastrointestinal Tracts of Large Mammals.** R. F. SHUMARD, I. A. SCHIPPER AND D. F. EVELETH, North Dakota Agricultural Experiment Station.

This device permits the rapid and accurate evaluation of the helminth population of large animals. It is extremely useful where large numbers of experimental animals are slaughtered or in survey work.

91. **Studies on the Susceptibility of Hamsters to** *Trichinella spiralis*. ELVIO H. SADUN AND LOIS NORMAN.

Female hamsters of the same age were inoculated with graded single doses of from 125 to 8,000 larvae of *Trichinella spiralis*. Up to a certain threshold, increases in the size of the inoculum resulted in a decrease in the percentage development of the larvae resulting therefrom. With doses of 500 larvae or more, however, infections overwhelmed the resistance of hamsters and the ratio of developing larvae increased significantly.

With inocula of 4,000 larvae, the greatest amount of worm elimination took place between the second and third day and with an inoculum of 8,000 larvae, the greatest elimination had already occurred two days after feeding of the larvae.

The $LD_{50}$ of hamsters of the age and size used was approximately 850 larvae. Death from a single heavy dose occurred primarily at the time of the intestinal phase in massive infections (4,000 larvae or more) and at the time of the muscular phase in lighter infections (3,000 larvae or less). Animals receiving 4,000 larvae or more lost weight almost immediately whereas those receiving 500 and 3,000 larvae gained weight during the intestinal phase and began losing it during the muscular phase. A correlation between the size of inoculum, the number of animals which died in each group and/or the time of death was apparent. The regularity of these results suggests the possibility of using lethal infections as an additional tool for the determination of the degree of resistance in different groups of hamsters.

92. **Establishment of** *Trichinella spiralis* **Infections in Thiry-Vella Fistularized Rats.** CHAUNCEY G. GOODCHILD; AND WESLEY BOODISH, Emory University.

White rats had their small intestine exposed, and a section, with blood vessels intact, resected. The main body of intestine was rejoined, and the resected section sewed to the flank to form a U-shaped isolated piece open at each end to the surface, properly called a Thiry-Vella fistula. Rats were normal in color, behavior and vigor after recovery.

Two hundred artificially excysted *Trichinella spiralis* larvae, free from exogenous food, were implanted deep into the fistula of 6 experimental rats, and 200 larvae were fed orally to each of 2 operated and 4 non-operated control rats. Bits of muscle were examined for juveniles 4–5 weeks after infection by both compression and artificial digestion. Total counts of juveniles digested from animals in the experimental series ranged from 74–1475.

Total counts of juveniles in orally-fed controls all were well over 5000. Larvae recovered from experimental animals yielded typical infections when fed to uninfected animals.

*Trichinella spiralis* has proved capable of extracting sufficient nutrients from host tissue or tissue secretions to mature and reproduce in an enteric environment free from exogenous food.

93. **Further Observations on Phthalofyne as a Trichuricide.** FRANK EHRENFORD, GEORGE BURCH, AND CARL BUNDE. Research Department, Pitman-Moore Co., Indianapolis, Indiana.

Previous investigations (Ehrenford 1954, J. Parasit. **40** (5 Sect. 2): 481) showed the marked specificity of phthalofyne for *Trichuris vulpis* in dogs when administered orally. Another route of administration, the intravenous, has been found to retain anthelmintic efficacy. The intravenous dose required for the removal of *T. vulpis* is 1.6 greater than the oral dose, when compared on the basis of a single therapeutic dose.

94. **The Storage and Standardization of Sera and Reagents for the Diagnosis of Trichinosis by the Bentonite Flocculation Test.** LOIS NORMAN, CDC., Chamblee, Georgia.

Bozicevich and his co-workers (1951) developed a practical and specific flocculation test for the diagnosis of trichinosis by adsorbing crude larval extract onto stock bentonite. To facilitate the widespread use of this test by small laboratories, an attempt has been made to standardize and store the various reagents for general distribution. An acid soluble protein fraction which could be lyophilized and stored over long periods of time was prepared (Sadun and Norman, 1955).

Batches of standard-sized bentonite particles were suspended in 4 ml. of glass distilled water. One half of each batch was sealed in glass vials and stored at 4° C. and the other half was lyophilized and stored in powder form. Both portions were sampled, at regular intervals, for antigen adsorption. The portion stored with water retained its properties for three months but was unsatisfactory afterwards. The lyophilized portion, after several months, has not yet lost its ability to adsorb antigen and is producing similar results when tested with standardized sera.

Preparations of bentonite particles already coated with the antigen did not retain their properties when lyophilized either before or after introduction of tween 80.

Sera of known titers and negative controls from humans, hogs and rabbits have been lyophilized, stored in glass vials at 4° C. and tested at regular intervals for several months. Results, to date, have shown no significant changes in their antibody titers. However, after lyophilization, the hog sera, when reconstituted, produced flocculations with the bentonite test that were difficult to read.

95. **Determinations of the Phosphorus Content of Golden Nematode and Root-Knot Nematode.** V. H. DROPKIN, The U.S. Nematode Research Lab., Seaford, New York.

The phosphorus content of two species of plant parasitic nematodes was measured as the first step in a program designed to establish the influence of certain nematode infections on the mineral nutrition of the host plant. Tomato plants were uniformly labelled with P-32 in sand cultures. Plant parasitic nematodes (*Heterodera rostochiensis* and *Meloidogyne incognita acrita*) were added and subsequently, samples of infected roots were taken at intervals for determinations of the phosphorus content of the nematodes, measured by radioactivity. Volumes of these nematodes were calculated from photographs. Results of determinations on 257 individual organisms of both species showed that the amount of phosphorus per nematode rose from about 0.0002 microgram in 2d stage larvae to about 0.1 microgram in adult females. These values range from 0.1% to 0.2% phosphorus in both species studied.

96. **The Life History of *Toxascaris leonina* in the Cat.** J. F. A. SPRENT, University of Queensland, Brisbane, Australia.

Cats were fed with embryonated eggs of *Toxascaris leonina* originally collected from *Lynx canadensis;* the cats were killed at intervals after infection. At 5 days a single second stage larva was found in the intestinal wall with a length of 0.278 mm. At 7 days many larvae were found in the wall and contents of the intestine; their length was 0.333–0.507 mm.; some were moulting at 0.435–0.471 mm. At 11 and 14 days many larvae, apparently in the third stage, were found in the same location; the length was 0.514–0.643 mm. At 21 days moulting larvae measuring 0.613–0.654 mm. were present, as well as many third and fourth stage larvae. The latter were distinguished by their well-defined lips, although their length at this stage was no greater than the third stage larvae. At 28 days the length of the fourth stage

larvae was 0.650–2.667 mm. Infections of longer duration showed that the fourth moult occurred at a length of 5.911 mm.; sexual differentiation occurred during the fourth stage and eggs first appeared in the faeces at 10.5 weeks.

Mice were infected with eggs and the larvae in the tissues grew from an average length of 0.225 mm. on the first day to 0.567 mm. at 4 weeks; a moult was not observed. Infected mice were fed to cats; adult specimens were recovered 35 days after infection.

97. **The Life-History of *Toxacara cati* in the Cat.** J. F. A. SPRENT, University of Queensland, Brisbane, Australia.

Embryonated eggs containing second stage larvae were fed to cats. The larvae reached the liver, lungs and muscles; the second moult occurred in the lungs at a length of 0.360 mm. about 5 days after infection, but no growth occurred except in those larvae which reached the stomach wall. The larvae grew to a length of 1.0 mm. and completed the third moult about 19 days after infection. Granulomatous cellular clusters around the larvae in the mucosa were visible at this time. Fourth stage larvae passed into the stomach contents and thence into the intestine where they grew to a length of 5–6 mm. and moulted for the fourth time. Mature females were present at 6 weeks. In mice the larvae migrated to the liver, lungs and somatic tissues but did not reach the alimentary tract. The second moult was observed about 10 days after infection but no growth in length occurred beyond 0.453 mm. Eggs were fed also to sheep, cockroaches and earthworms. In each animal larvae were recovered from the tissues and in sheep muscle the second moult was observed. When infected mice were fed to cats the larvae did not migrate into the somatic tissues but directly entered the stomach wall where they commenced development as in egg-infected cats. The migratory tendencies of the second stage larvae were evidently fulfilled in the mouse, with the result that in mouse-infected cats development of the larvae occurred more rapidly than in egg-infected cats.

98. **The Life History of *Ophidascaris filaria* in the Carpet Snake (*Morelia argus*).** J. F. A. SPRENT, University of Queensland, Brisbane, Australia.

The adult parasites congregate in clusters with the anterior part of the body deeply buried in the stomach wall. The eggs are spherical, with deeply pitted shell; they measure approximately 0.085 mm. in diameter and pass out in the feces. Development proceeds only in well-aerated cultures, the first stage larva being completed in 10 days; the first moult occurs at this time and the eggs are then infective to mice. In mice the second stage larvae migrate to the lungs and general musculature where after 5 days, some undergo the second moult, at a length of 0.5–0.6 mm. Third stage larvae appeared in the liver at 5 days and thereafter grew considerably, reaching a length of 12 mm. by the fourth week; fatal liver damage frequently resulted; larvae in other tissues reached only 1.7 mm. Carpet snakes were fed living infected mice and killed at various intervals after infection. Third stage larvae were found in the kidneys, liver and lungs, measuring up to 11 mm. The third moult was observed in the lungs. Fourth stage larvae, and adults with the fourth stage cuticle still attached, were found in the stomach wall three weeks after infection.

99. **The Effectiveness of Piperazine Citrate against Intestinal Parasites, Especially *Ascaris*, in Mental Patients.** MARTIN D. YOUNG, DHEW, PHS, National Institutes of Health, NMI, Laboratory of Tropical Diseases, Columbia, South Carolina; AND JOE E. FREED, South Carolina State Hospital, Columbia, South Carolina.

Because the number and intensity of parasitic infections constitute a serious problem in mental hospital patients better methods of treatment are being sought. Piperazine citrate was tried in several regimens. The results with *Ascaris* and *Enterobius* infections are shown in the following table:

| Drug given—grams | | | Patients with: | |
|---|---|---|---|---|
| No. of days | Daily Dose | Total | *Ascaris* After Rx/Before Rx | *Enterobius* After Rx/Before Rx |
| 1 | 1 | 1 | 1/1 | |
| 1 | 3 | 3 | 2/3 | |
| 2 (on ea. of two weeks) | 2 | 8 | 1/2 | 0/1 |
| 4 | 2 | 8 | 1/2 | 1/2 |
| 4 | 3 | 12 | 11/15 | 0/1 |
| 7 | 2 | 14 | 0/9 | 1/3 |
| 10 | 2 | 20 | 0/3 | |

The regimens of 2 grams daily for 7 and 10 days were effective against *Ascaris* infections. No untoward results were observed. A syrup preparation of the drug was used in all but three cases, where tablets were used; no difference was observed between the effectiveness of the two preparations.

Some *Enterobius* infections were eliminated but the drug action was not consistent.

Of four *Giardia* infections, one was eliminated with an eight-gram regimen but the other three were not eliminated by higher dosages. The drug did not cure the following number of parasitic infections: *Entamoeba coli*, 28; *E. nana*, 2; *Iodamoeba*, 3; *Trichomonas hominis*, 11; *Chilomastix*, 2; *hookworm*, 38; *Strongyloides*, 26; *Trichuris*, 39.

100. **Piperazine Citrate as an Anthelmintic in Domestic Animals.** R. F. SHUMARD AND D. F. EVELETH. North Dakota Agricultural Experiment Station.

Experimental evidence and field trials indicate high anthelmintic properties of piperazine citrate against *Ascaridia galli*. The drug was effective against this roundworm at dosages of 4000 mg. per gallon of drinking water. Removal of adult and immature forms was greater than 95 percent and occurred within six hours. Experiments with dosages of 2000 mg., 1000 mg., and 500 mg. per gallon of drinking water showed that although some removal was effected, efficiency was not as good at these lower levels. However, continous preventative doses as low as 1000 mg. per gallon of water are effective in preventing infection with *A. galli*.

Preliminary studies indicate the effectiveness of the drug against *Ascaris lumbricoides* in swine and *Toxascaris leonina* in dogs. Studies of the effectiveness of piperazine citrate on helminths of sheep are inconclusive.

101. **The Value of Repeated Examinations in the Diagnosis of Infections with** *Enterobius vermicularis*. ELVIO H. SADUN AND DOROTHY M. MELVIN, CDC, Chamblee, Ga.

A total of 1,842 cellulose tape preparations were collected on 6 consecutive nights from 307 patients in a mental institution. The prevalence rate of *Enterobius vermicularis* for the entire group was 67 percent. If only a single specimen had been collected, the prevalence rate would have been between 25 and 37 percent. The probabilities of demonstrating infection increased somewhat regularly with each consecutive examination. After the third examination, eggs had been found in 90 percent of the estimated infected population.

The efficiency of consecutive examinations was compared in population groups with different prevalence rates. Sixty percent of the positives were detected by the first examination in a heavily infected group whereas 37 percent were detected in groups with a lower prevalence rate. After the third examination, eggs had been found in 91 and 90 percent of the positives in the respective groups.

The findings indicate that a single examination reveals only a fraction of the existing infections and does not permit an estimation of the true incidence of infection. The differences in demonstrability in population groups with varying incidences largely disappear after the third examination and the results are thus directly comparable. A system of six consecutive examinations may be necessary if a high and constant proportion of the existing infections is desired.

102. **Proteolytic Activity in Extracts of the Esophagus of Adults of** *Ancylostoma caninum* **and the Effect of Immune Serum on This Activity.** R. E. THORSON, Alabama Polytechnic Institute.

Physiological saline extracts of the esophagus dissected from adults of *Ancylostoma caninum* showed proteolytic activity against casein. The pH optimum of this activity was approximately 6. Since it has been postulated that enzymes necessary to the physiology of the worms serve as antigens and that antibodies to these enzymes inhibit their activity thereby making the environment of the host unsuitable for the worms, experiments were designed to test this hypothesis. When serum from a dog which had been infected periodically for 4 years with larvae of *A. caninum* and was refractory to further infection with *A. caninum* was added to the esophageal extracts before the addition of the substrate casein, it was found that this immune serum inhibited the proteolytic activity by as much as 88 percent when compared to preparations in which normal serum or saline were added to the extract. (This investigation was supported in part by a research grant E-872 from the National Microbiological Institute of the National Institutes of Health, Public Health Service.)

103. **The Effect of Extractions of the Amphidial Glands, Excretory Glands and Esophagus of Adults of** *Ancylostoma caninum* **on the Coagulation of Dog's Blood.** R. E. THORSON, Alabama Polytechnic Institute.

That substances from *Ancylostoma caninum* inhibit the coagulation of blood from a dog has been known for many years (Loeb and Smith, 1904, Zentr. Bakt. Parasitenk. Abt. 1., Orig. 37: 93-98). Adults of the hookworm were dissected and the amphidial glands, excretory glands and esophagus were isolated. Physiological saline extracts of each were tested in an isolated thrombin-fibrinogen clot reaction. None of the extracts delayed coagulation. When they were used in a prothrombin-time determination, it was found that as little as 50 microliters of amphidial gland extract mixed with thromboplastin before the addition of plasma delayed the coagulation by as much as 100 percent. In addition, the clot formed was never complete as compared with preparations in which saline was used instead of extract. The substance causing this delay is heat stable and is inhibited somewhat by both normal or immune serum, but the clot formed in these preparations was never complete. (This investigation was supported in part by a research grant E-872 from the National Microbiological Institute of the National Institutes of Health, Public Health Service.)

104. **Evaluation of the Reliability of the Dilution Egg Count and the Direct Smear Egg Count in the Quantitative Determinations of Hookworm Infections.** DOROTHY M. MELVIN, ELVIO H. SADUN, AND ROGER HEIMLICH, CDC, Chamblee, Ga.

Fecal samples from humans, dogs and rats were used for a comparison of the variability of the Stoll and the Beaver egg count techniques. A total of 760 counts were done on 30 specimens. The "coefficient of dispersion" (the variance divided by the mean) was used to determine the reliability of the two methods.

In human specimens, very little deviation from a random distribution was found with both techniques. However, a statistically significant "over-dispersion" was observed with the direct smear egg count in a few samples with a high number of eggs. No evidence of person to person variation was observed when different investigators examined the same specimen with both techniques. In samples from dogs and rats, a significantly greater variance was observed. In all the specimens from man and lower animals, the departure from the random distribution always increased with the size of infection.

These results suggest that both techniques can be effectively employed in estimating the magnitude of hookworm infection in humans. The direct smear egg count is regarded as the method of choice for routine quantitative diagnostic purposes in view of the fact that the performance time is approximately one-half that required for the Stoll technique. If, under special conditions, a relatively more accurate determination is necessary for research purposes with stools of dogs and rats, the dilution egg count might be preferable.

105. **The Occurrence of a Protostrongylid Nematode in the Meninges of Michigan Deer.** DOMINIC L. DEGIUSTI, Wayne University, Department of Biology.

Examination of 79 deer heads revealed an infection rate of 46% with a Protostrongylid nematode in the meninges. The nematodes are localized on the inner surface of the meninges, may project onto the brain surface, and are widely distributed in the cranial cavity.

No fully mature worms have been found, but all worms are well advanced in development toward maturity. The presence of worms in the meninges elicits an inflammatory reaction accompanied by bleeding in the surrounding area.

106. **Some Nutritional and Physiological Changes Taking Place in Lambs Infected with *Haemonchus contortus*, *Trichostrongylus colubriformis*, and *Nematodirus spathiger*.** R. F. SHUMARD AND D. F. EVELETH. North Dakota Agricultural Experiment Station.

Eight lambs, raised parasite free, except for a few *Strongyloides*, were placed in two different lots and fed the same rations. Four of the lambs were given 100,000 *Trichostrongylus colubriformis*, 40,000 *Haemonchus contortus* and 10,000 *Nematodirus spathiger* per lamb. All infected lambs died within 27 days following infection. Much variation in the number and species of worms recovered at necropsy occurred, one lamb having no *Haemonchus* present. Daily feed and water consumption was much less in the infected lambs. 24-hour fecal samples, analyzed for water content, varied according to the type and course of the infection.

Blood samples, which were withdrawn every other day and analyzed, showed the following: Hemoglobin levels and hematocrit values dropped in all of the infected lambs except the one having no *Haemonchus* at necropsy. Inorganic phosphorus levels dropped more than 50 percent in all infected lambs and then made slight recoveries. Blood glucose levels of the infected lambs fluctuated greatly with a tendency towards a higher level. Total serum protein varied from time to time in the infected animals but did not decrease nor increase consistently. Serum albumin decreased consistently, with a corresponding increase in serum globulin.

At the time of the preparation of this abstract data on differential white blood cell counts,

pH of the feces and digestibility of protein, crude fiber and phosphorus are not complete but will be presented.

107. **Effect of Two Rations, Differing Primarily in Protein, Carbohydrate and Crude Fiber Content, on Experimental Haemonchosis in Lambs.** K. C. KATES AND G. I. WILSON, Animal Disease and Parasite Research Branch, Agricultural Research Service, U. S. D. A., Beltsville, Md.

An experiment was carried out to test the effects of two different rations on experimental haemonchosis in lambs. One feed used was high grade, chopped alfalfa hay and the other a pelletted ration developed for fattening lambs at State College, New Mexico, consisting of 60% alfalfa meal, 30% milo maize and 10% cane molasses. The alfalfa-grain-molasses ration was lower in crude protein and crude fiber, and higher in nitrogen-free-extract (soluble carbohydrate) than the alfalfa hay.

Four comparable groups of 3 lambs each were employed. Groups I and II were fed the pelletted ration and Groups III and IV the chopped alfalfa hay. After a 2-week, preliminary conditioning period on the two rations, lambs of Groups I and III were given 15,000 *Haemonchus contortus* larvae each; the other groups were uninfected controls. The experiment was terminated 11 weeks after larvae were given. The avg. feed consumed per pound of grain was as follows: Group I—6.36 lb.; II—5.98 lb.; III—7.97 lb.; IV—6.27 lb. Both the infected and uninfected lambs on the pelletted ration showed better feed utilization than the comparable groups on alfalfa hay. Also, the infected lambs on the pelletted feed had higher avg. hemoglobin levels, lower avg. fecal egg counts and lower post-mortem worm counts, than the infected lambs on alfalfa hay. Further evidence, therefore, is provided that lambs maintained on a high nutritional level show better utilization, less severe clinical effects and fewer worms than lambs on a lower nutritional level.

108. **The Relation of Pasture Rotation to Acquisition of Strongyline Nematodes by Sheep.** NORMAN D. LEVINE AND DAVID T. CLARK, University of Illinois.

In an experiment carried out between mid-April and October, 1954, a 4/5 acre alfalfa-rye pasture was divided into two halves, and one half was further divided into 6 lots. Three ewes and 3 lambs were kept in the large lot continuously, while 3 ewes and 3 lambs were rotated through the small lots on a weekly schedule, each lot being grazed for 1 week and rested for 5. The fecal strongyline egg counts of the ewes in both rotated and control lots remained low throughout the experiment. Strongyline eggs (mostly *Haemonchus contortus*, identified by larval cultures) appeared in the control lambs 35 days after they had been put on pasture, and in the rotated lambs 2 weeks later. They had reached peaks of 8844 per gram feces in the rotated lambs and 9900 per gram in the controls at the end of July, and then declined rapidly and spontaneously.

Although this rotation system did not prevent lambs from becoming heavily parasitized, its effect on pasture utilization was striking. The control pasture was completely grazed off after 57 days, and hay had to be fed the control sheep thereafter. The rotated pastures held up throughout the grazing period of 168 days. Thus the pasture lasted 3 times as long under rotation as without it.

109. **Some Preliminary Observations on the Life History of *Amidostomum anseris* Zeder, 1800.** ARCHIBALD B. COWAN, Patuxent Research Refuge, U.S. Fish and Wildlife Service, Laurel, Maryland.

The trichostrongyle *A. anseris* is a parasite of the gizzard lining of wild and domestic geese and ducks. It is common in Canada geese (*Branta canadensis*) and is thought to be a contributing factor to winter losses in some areas. Investigations conducted to determine causes of these losses have involved studies on the biology of this nematode.

The eggs of *A. anseris*, observed in fresh feces, had developed, in most instances, to the morula stage. Hatching occurred in 24 to 48 hours in tap water at room temperature. At 6 degrees centigrade embryonic development continued to completion, but the larvae remained inactive and failed to break out of the shell. When warmed to room temperature they hatched. The embryo developed as well in brackish water as in tap water.

Larvae were observed to shed their first sheaths at the time of hatching. The second sheath was then visible and did not appear to undergo subsequent change. It has not yet been determined when the larvae become infective; however, birds were infected with larvae obtained from six-day charcoal cultures. Larvae survived storage for a week or ten days in water at 6° C. without much loss in numbers or infectivity. When kept longer there was evidence that infectivity decreased. Some larvae were active after freezing and thawing.

Experimental infections were established per os. Time for worms to develop to maturity varied with age of the goslings. *Amidostomum* eggs were detected in the feces of some goslings as early as 14 days after exposure, while in others no eggs were found until the 25th day. All the parasites did not reach maturity simultaneously. Infections persisted for periods exceeding 18 months, though they frequently lessened in intensity.

Soil samples taken from a study area were negative for *Amidostomum* larvae. Examination of sample plots seeded with *A. anseris* eggs resulted in poor recovery of larvae. *Amidostomum*-free geese released on the same area in November and afforded freedom of movement and association with the wild wintering population became infected by the following March.

110. **The Role of the Skin and Lungs in the Development of** *Nippostrongylus muris*. DONALD W. TWOHY, The Johns Hopkins University.

Controlled experimental comparisons were made between larvae of *Nippostrongylus muris* injected subcutaneously into the skin and larvae carried to the lungs after injections into the femoral vein to determine the role of the skin in the development of the larvae. The recoveries of adults were slightly higher in the animals receiving subcutaneous injections, but this difference may have resulted from experimental bias. About 10 percent more of the larvae injected intravenously were present in the digestive tract at given intervals during the lung-digestive tract migration than larvae in animals injected subcutaneously. Development in the skin could constitute only 1 or 2 hours delay over initial development in the lung. Using the same methods of infection there was no difference in the size at 10 hours after infection between larvae from the skin and larvae from the lungs. From these observations it can be concluded that the skin is neither essential nor a marked handicap to larval development. The initial lag in growth is inherent in the larvae, not in the site of development.

Larvae injected into the liver via the portal vein appeared to undergo a normal development when compared to forms injected into the lungs via the femoral vein. Many of the larvae were able to pass through the liver to the lungs, but those that remained developed normally and were capable of growing to adults when fed to other rats. Therefore, the lung can not be considered the only tissue to meet the physiological requirements in the early growth of *N. muris*.

111. **The Morphogenesis of the Female Reproductive System of** *Skrjabinoptera phrynosoma* **Schulz, 1927, a Gastric Nematode of Texas Horned Toads** *Phrynosoma cornutum*. SHERIDAN H. LEE, Wayland College, Plainview, Texas.

The female reproductive system of *Skrjabinoptera phrynosoma* commences its development within the definitive host, the Texas horned toad, *Phrynosoma cornutum*. It first appears as an oval cell mass 0.033 by 0.029 mm. The location of the cell mass indicates the site of the vulva. Then the cell mass elongates and dichotomizes posteriad. The reproductive tubules average 0.033 mm. in diameter. When the ramus reaches about 6.5 mm. an enlarged chamber (0.264 by 0.66 mm.) appears 0.3 mm. posterior to the vulva. Then the ramus differentiates into ovary, oviduct, receptaculum seminis, and uterus. The enlarged chamber has been known as egg chamber. It should be called spermatheca. The appearance of reproductive ducts before the appearance of gonads is a unique type of morphogenesis as compared with higher forms of animals.

112. **The Effect of Previous Infection on Filarial Worms Transferred from One Cotton Rat to Another.** J. ALLEN SCOTT, ETTA MAE MACDONALD AND LEROY J. OLSON, The University of Texas Medical Branch, Galveston.

Cotton rats were infected with *Litomosoides carinii*. Seven days later the developing worms were recovered and transferred surgically to the abdominal cavity of two other groups of cotton rats. The rats of the first group had each received three infections at approximately weekly intervals 30 to 50 days previously, while the second group consisted of uninfected controls. All of these rats were killed 17 days later, i.e., when the transferred worms were 24 days old. The worms from the control rats were not significantly longer than those from the experimental rats and no greater percentage of worms had entered the fourth molt. Previous experiments have shown that worms growing the entire 24 days in immunized rats are smaller than those from similar control rats. Therefore, it is concluded from the present experiment that the depression of growth rate resulting from immunizing infections must be effected during the first seven days.

113. **Infection of White Rats with the Filarial Worms of Cotton Rats.** LEROY J. OLSON, J. ALLEN SCOTT, AND ETTA MAE MACDONALD, The University of Texas Medical Branch, Galveston.

Previous reports have indicated that the rat is partially immune to the filarial worms (*Litmosoides carinii*) of the cotton rat. Preliminary experiments indicate that this immunity is more pronounced in older white rats than in white rats only a few weeks old. An attempt has been made to determine whether white rats under treatment with cortisone have less immunity than similar untreated rats, as is the case with certain other species of nematodes which infect by skin penetration. Preliminary results indicate that at least in older rats cortisone has no such effect. It is suggested that in this species the immunity may not be the result of a reaction in the skin of the host.

114. **Black Flies (Simuliidae) as Vectors of** *Ornithofilaria fallisensis* **Anderson, 1954.** Roy C. Anderson, Department of Parasitology, Ontario Research Foundation, Toronto 5, Ontario.

It was reported earlier that *Mf. fallisensis* would develop to the infective stage in *Simulium venustum* Say. Further studies show that development is completed also in *S. parnassum* Mall., *S. rugglesi* Nicholson and Mickel, and in others tentatively identified as *Eusimulium euryadminiculum* Davies, *E. croxtoni* Nicholson and Mickel and *E. latipes* Meigan. Regular catches of flies off domestic ducks during the spring and summer of 1955 in Algonquin Park, Ontario yielded all these species of black flies except *S. venustum* and *S. parnassum*. *Eusimulium* spp. started feeding on the ducks about May 17th; *S. rugglesi* on May 26th. *Eusimulium* spp. were not collected off ducks after June 15th but *S. rugglesi* was active up to July 25th. Ducklings exposed from May 17th–July 2nd, when these flies were active, became infected. It is concluded therefore that *Eusimulium* spp. were the early vectors of *O. fallisensis*, but later the sole vector was *S. rugglesi*. Adult *O. fallisensis* have been collected from a wild black duck (*Anas rubripes*) from Algonquin Park, and it is believed that these ducks are the usual hosts of this filarioid.

115. **Current Status of Intestinal Parasitism of Man in Eastern Kentucky.** F. O. Atchley, E. C. Hemphill, and D. W. Hunt. From the Communicable Disease Center, Public Health Service, U. S. Department of Health, Education, and Welfare, Prestonsburg, Kentucky.

Parasitological observations were made on stool specimens from populations in 10 study areas. The investigations were conducted among inhabitants of mining camps and rural hamlets in the Eastern Kentucky mountains of the Cumberland Plateau. Single fecal samples were collected at seasonal intervals and laboratory examinations were directed routinely toward detection and identification of intestinal protozoan cysts and helminthic ova. In a relatively few selected instances, specimens were obtained in a manner permitting observations of trophozoites if present. Results of some 3000 observations on 2200 individuals including all ages were tabulated. Approximately one-third of the population harbored one or more intestinal helminths, principally *Ascaris lumbricoides* and *Trichuris trichiuria*. *Enterobius vermicularis* was reported highly prevalent, but this parasite is not reliably observed by the routine fecal examinations performed. Rates of protozoan infections did not exceed those found in other regions.

116. **Some Problems in Parasite Control in Southern Brazil.** James E. Ackert, Kansas State College, Manhattan, Kansas; and Uriel F. Rocha, University of São Paulo, Brazil.

Topics to be discussed briefly are those related to (a) Climate—temperature and moisture; (b) Soil and food deficiencies—minerals and vitamins; (c) Personnel—scarcity and multiple duties; and (d) Screens—high or prohibitive cost.

117. **Some Helminthological Problems in a Zoological Park.** Benedict J. Jaskoski, Chicago Zoological Park and Loyola University (Chicago).

Direct observation and flotation technics were employed in the examination of over 1,000 fecal samples from captive reptiles, birds, and mammals. Heaviest individual infections by intestinal helminths were found among some mammals recently arrived from their native environments. The highest percentage of infection among those animals observed was by strongyle species in ruminants. Particular problems of strongyliasis in the giraffe and the Okapi are discussed. The roles of nutrition and infections other than helminth as they effect helminth infection are considered.

118. **Incidence of Poultry Parasites under Different Ecological and Geographical Conditions in Egypt.** W. Malcolm Reid, University of Georgia.

Little information has been available as to the incidence and thus the economic importance of various species of poultry parasites in Egypt. Among the helminth parasites, *Ascaridia galli* constitutes the most serious problem causing losses and lack of normal weight gains. The seriousness of this parasite is aggravated by irrigation practices and methods of water use around homes and chicken yards since all damp areas suitable to worm-egg germination are man-made. *Raillietina echinobothrida* was found in 28 percent of fowls surveyed indicating a serious problem since this species causes nodular pathology in the gut.

*Argas persicus* is the most serious arthropod parasite. Although it is distributed widely in Egypt, it constitutes much more of an economic problem among small farmers in Upper Egypt than in the Cairo and Delta areas. The ticks are found in 80 percent of the village homes in the Assiut area and is known and recognized by every farmer. In Cairo and Delta areas, less than one percent of the village homes have tick problems and most of the farmers do not recognize the chicken tick. The use of modern insecticides together with enforcement of strict isolation can effectively control this parasite. Serious losses are realized through the protozoan diseases of coccidiosis and spirochaetosis. The former can be controlled through the use of coccidiostatic drugs while the latter requires tick control and chemotherapy.

Two species of cestode, *Raillietina cesticillus* and *Hymenolepis carioca* and one species of mite, *Bdellonyssus bursa* are reported from Egypt for the first time.

119. **Notes on Animal Filarial Parasites in Malaya, with Special Reference to** *Macaca cynomolgus*. DONALD L. PRICE, Army Medical Service Graduate School, Washington, D. C. AND LEO A. JACHOWSKI, Naval Medical Research Institute, Bethesda, Maryland.

In the spring of 1955, in cooperation with the Filariasis Research Laboratory of the Institute for Medical Research, the authors made a survey of the filariae in animals collected in the vicinity of Kuantan, Malaya. A total of 234 animals was examined. Microfilariae were found in 9 of 21 species represented. The infected species include Malayan domestic fowl (*Gallus sp.*), barred ground dove (*Geopelia striata striata*), Malayan domestic dog (*Canis familiaris*), Prevost's squirrel (*Callosciurus prevostii*), three species of rats (*Rattus annandalei, R. exulans,* and *R. rattus jalorensis*), slow loris (*Nycticebus coucang*), and the long-tailed macaque (*Macaca cynomolgus*). The barred ground dove was found to harbor two species of microfilariae. Adult filariae of one of these were removed from the subcutaneous connective tissue of one infected dove at post-mortem examination. Adult filariae were recovered from the pleural and peritoneal cavity of the three species of rat. These adult worms belong to the same genus and probably the same species. Microfilariae of the two species were found in *Macaca cynomolgus,* and one species of adult belonging to the genus *Dirofilaria* was recovered from the subcutaneous connective tissue.

One of the microfilariae in the macaque was sheathed and indistinguishable from microfilariae in humans in that area. This has been previously reported by Poynton and Hodgkin in 1938. On the basis of microfilarial morphology, the species in humans is considered to be *Wuchereria malayi*. Mosquitoes of the genus *Mansonia* were fed on infected monkeys and humans. The larvae which developed in the mosquitoes could not be separated on the basis of morphology, staining characteristics, site in mosquito, or length of time for development. Adults of this species were not obtained.

120. **The Extra-Corporeal Hatching of the Eggs of** *Trichuris vulpis*. LOPE M. YUTUC, University of the Philippines.

The eggs of *Trichuris vulpis* were collected in sufficient amount from the excrement of an infected dog by sedimentation and flotation using super-saturated salt solution. These were placed in six-centimeter-diameter Petri dishes with tap water for embryonation. The water was changed at frequent intervals. At least three days were allowed to pass before using the embryonated eggs for hatching purposes. Paired Petri dishes with sufficient number of embryonated eggs were used for each trial. The tap water medium was carefully pipetted from each Petri dish and replaced with Locke's isotonic solution in the amount of 5 cc. for each culture. In one Petri dish 3 drops of dog bile was added, stirred and incubated along with the control at 37 to 39° C. Ten trials were carried. Observations were made daily with the low power objective of a compound microscope.

In the first trial 25 percent of the embryonated eggs hatched in the bile-Locke's solution; in the second 28 percent, in the third 26 percent, in the fifth 1 percent, in the sixth 4.8 percent, in the fourth and the seventh to the ninth none hatched, and in the tenth three percent. Only in one control dish was one empty egg-shell noted in the series.

In some of the trials the pH of the cultures was checked. In those that failed to hatch, the pH was 5.6 for the culture with bile and 6.7 for the culture with Locke's solution alone. In those, where hatching took place the pH was 6.0 and the control 6.8.

The earliest hatching was noted two hours after incubation. In hatching the embryo emerged through one of the plugged poles of the egg. As a rule the following day all larvae were found dead. With the addition of dextrose in 5 percent concentration to the culture, the life of the larvae was extended to six days. The larvae measured on the average 0.31 by 0.01 mm.

121. **Cellular Factors in Acquired Immunity to *Trichinella spiralis*, as Indicated by Cortisone Treatment of Mice.** Coit M. Coker, University of North Carolina School of Public Health.

In an experiment involving 92 mature white mice of the same age, 76 were immunized by giving them three stimulating infections with 200 *T. spiralis* larvae, spaced 21 days apart. One month after the time of the last immunizing infection, when the mice were about 18 weeks old, all except 8 mice (immunized persistency controls) were challenged with 400 larvae. Thirty-four of the immunized mice were given daily subcutaneous injections with 0.5 mg. of cortisone beginning 3 days prior to the challenging infection and continuing to necropsy. Statistical analyses of adult worm counts from the small intestines of 16 immunized mice, 8 persistency controls, 16 non-immunized controls and 16 cortisone-treated experimentals, at 7 and 14 days following the time of the challenging infection, indicated that the immunization procedure was successful in establishing immunity, and that the cortisone treatment destroyed the established immunity.

A histological study of the cellular reaction in the small intestine was performed on 36 of the immunized, challenged mice, half of which had received the cortisone treatment. Two mice from each of these two groups were sacrificed for this study at the following time intervals after the challenging infection: 12 hours, days 1, 2, 3, 4, 5, 6, 7, and 9. The cortisone treatment eliminated the cellular reaction in the intestinal wall which customarily occurs just prior to the operation of acquired immunity.

The results of the experiment, when considered in the light of additional evidence drawn from the literature, are in harmony with the hypothesis that the immunity mechanism against this parasite in mice is due to the primary action(s) of specific antibodies with secondary cellular cooperation. However, the results seem to indicate that the cellular factor is of greater importance than the serologic factor(s) in bringing about the actual elimination of the worms from the small intestine. The mechanism by which this action occurs is speculative.

122. **Further Studies on Plant-Parasitic Nematodes Maintained in Altered Oxygen Tensions.** William A. Feder and Julius Feldmesser, Fruit and Nut Crops Section, Orlando, Florida and Nematology Section, Sanford, Florida, respectively, Agricultural Research Service, United States Department of Agriculture.

Work reported previously, (Feldmesser & Feder, 1954, J. Parasit. **40** [5, Sect. 2], 18), has been continued to include studies in Florida on the effects of increased oxygen tensions on *Hoplolaimus coronatus, Radopholus similis, Belonolaimus gracilis, Tylenchulus semi-penetrans, Dolichodorus heterocephalus.* Increased oxygen tensions were produced by 400 ppm of sodium sulfite in sealed tap water solutions containing the nematodes. Immersion in these solutions resulted in reduction and finally, cessation of motility. *Hoplolaimus* lost motility in 5 to 7 minutes, *Radopholus* in 7 to 9 minutes, *Belonolaimus* in 10 minutes, *Tylenchulus* in 10 to 12 minutes and *Dolichodorus* in 45 minutes. Resumption of motility after aeration of the solution was immediate for *Hoplolaimus* and *Belonolaimus* within 5 to 10 minutes for *Radopholus* and *Dolichodorus* and 90 minutes for *Tylenchulus.* Recovery of motility occurred within 15 minutes of aeration after 24 hour periods of immobilization for *Belonolaimus* and *Dolichodorus* and within 30 minutes for *Hoplolaimus* and *Radopholus. Tylenchulus* again required 90 minutes.

123. **Further Studies on the *In Vitro* Culture of the Larvae of *Ascaris lumbricoides suum.*** T. D. Pitts, Loyola University of Los Angeles; and G. H. Ball, University of California at Los Angeles.

Longevity of newly hatched *Ascaris larvae* was extended beyond the 39 days reported previously (Pitts and Ball, 1953). The original medium, containing yeast extract, peptone, glucose and human serum in Fenwick's saline solution, was tested further at 37° C. and found to support up to 36.5% survival at 32 days and 1.6% at 44 days. At 30° C. up to 88.2% were active on the 32nd day, and 6.4% on the 81st day, while at 25° C. 65.6% were active on the 32nd day, and 20.7% on the 100th day.

Substituting hog serum for human serum and adding an extract of raw hog liver (RLE) further increased the longevity of larvae. At 37° C. up to 34.8% lived 40 days and 4.4%

were active to 64 days. At 30° C. there was survival up to 76.2% on the 41st day and 4% on the 102nd day.

Deficiency tests indicated that yeast extract, glucose and serum were essential, while larval survival was unaffected by absence of peptone or Fenwick's solution.

A continuous flow of fresh medium or renewal of the medium at 24 hour intervals did not appear to increase longevity.

Increases in length did not exceed 30%, however media of hog serum plus chick embryo extract (CEE), or Fenwick's saline plus CEE produced some larvae with increased length and diameter. Volumetric increases of approximately 100% occurred. Photomicrographs suggested some internal development.

Although some sheaths appeared to be loosened, no complete exsheathing was observed.

124. **On the Occurrence of Certain High Energy Phosphate Compounds in Filariform Larvae of** *Strongyloides ratti.* C. A. JONES, Veterans Administration Hospital; J. C. SWARTZWELDER, Louisiana State University School of Medicine; S. H. ABADIE, Veterans Administration Hospital, New Orleans.

Filariform larvae were grown in cultures of feces from rats infected with *Strongyloides ratti.* Bacterial growth in these fecal cultures was suppressed by mixing a combination of several antibiotics with the fecal culture. After incubation under standardized room conditions for 48 to 72 hours the filariform larvae were harvested, washed in distilled water and while still alive, quickly frozen in liquid nitrogen. They were stored at −15° C.

Because of the small size of samples which were obtainable by these methods, isolation and absolute identification of the various compounds was not feasible. However, analyses of barium fractionated trichloracetic acid extracts of the larvae furnished indirect evidence of the presence and the amounts of certain of the important high energy phosphate compounds and their degradation products. Consistently in all samples analyzed an average of 260 micromoles of a "labile phosphorus" was found, indicating the presence of a "phosphagen." This presumably is arginine phosphate in the absence of demonstrable free creatine in the extracts.

The presence of ATP, ADP and AMP was indicated by characteristic paper chromatogram. From analyses of the barium fractionated extracts approximately 600 micromoles per 100 grams of larvae of these compounds were present in approximate ratio of 2:1:2 (ATP:ADP:AMP).

It would appear from these studies that the filariform larvae of *S. ratti* in common with other living organisms has available the usually employed compounds for conversion of chemical energy to muscular work. In contradistinction to certain other parasitic helminths, this organism appears to utilize a storage reservoir for high energy phosphate in the form of a "phosphagen." (This investigation was supported by research grant E-543 from the National Microbiological Institute, of the National Institutes of Health, Public Health Service.)

125. **On the Occurrence of Glycogen and Phosphate Esters in Filariform Larvae of** *Strongyloides ratti.* C. A. JONES, Veterans Administration Hospital; J. C. SWARTZWELDER, Louisiana State University School of Medicine; S. H. ABADIE, Veterans Administration Hospital, New Orleans.

Filariform larvae grown in cultures of the feces of rats infected with *Strongyloides ratti* were harvested after incubation under standard conditions for 48 and 72 hours. Bacterial growth in the fecal cultures was supressed by admixture with a combination of several antibiotics. Collected larvae, while still alive, were washed in distilled water and frozen in liquid nitrogen. The frozen samples were stored at −15° C. until analyzed.

Because of the small size of samples obtainable, isolations of compounds and absolute identification was not possible. However, a combination of paper chromatography and analyses of trichloracetic acid extracts fractionated with barium by conventional methods furnishes convincing evidence of the occurrence of certain phosphorylated carbohydrates. Hexose phosphates were found to be present in the amount of approximately 1500 mircomoles per 100 grams of larvae. Of the hexose phosphates the individual esters occurred in the ratio of 8:6:1 (Glucose-6-Phosphate: Glucose-1-Phosphate: Fructose-6-Phosphate). Very small quantities of hexose diphosphate and triose phosphates were present; however, approximately 480 micromoles of 3-phosphoglyceric acid were found. The end product of glycolysis was apparently lactic acid which averaged about 460 micromoles per 100 grams of larvae and in individual analyses was roughly related to the percentage of ATP present. Analysis of the residue of the larvae after trichloracetic acid extraction showed that approximately 0.5% of the wet weight of the larvae is glycogen. The occurrence of these phosphate esters suggests that the usual glycolytic mechanisms obtain in this organism. However, the large amount and some-

what anomalous distribution of the various hexose phosphates is at present unexplained. (This investigation was supported by research grant E-543 from the National Microbiological Institute, of the National Institutes of Health, Public Health Service.)

126. **Parasitologic Studies in the Far East. XV. A Preliminary Survey for Intestinal Parasitism in Southern Formosa.** LYMAN P. FRICK AND ARTHUR P. MOON, 406th Medical General Laboratory, APO 500, San Francisco, California.

A group of approximately 1100 Chinese Nationalist troops from southern Formosa were examined for intestinal parasites by qualitative and quantitative methods on two occasions (October, 1954 and February, 1955). For comparison a single examination by similar methods was made on a group of 725 young Taiwanese males from the same area. Results of examinations show that while parasitoses are extremely prevalent, the intensity of the common helminthiases tend to be light. Hookworm was the predominant parasite, being found in 78.6 percent of the Chinese and 93.5 percent of the Taiwanese. *Ascaris lumbricoides* occurred in only 19.7 percent of the Chinese compared to 72.3 percent in the Taiwanese. Rates for *Trichuris trichiura* were respectively 58.8 and 89.0 percent. Nine infections with *Schistosoma japonicum* were observed in the Chinese; none was found among the Taiwanese. It can be assumed that the schistosome infections were contracted on the Chinese mainland and were at least of five years' duration. The *Endamoeba histolytica* rates were 23.9 and 27.7 percent respectively in the Chinese and Taiwanese. Appreciably fewer infections with *E. coli* were found, the rates being 15.3 and 14.7 percent. *Endolimax nana* occurred in 22.5 percent of the Chinese and only 15.9 percent of the Taiwanese. The unusual ratio between the frequency of the three amebas suggests the involvement of factors not previously recognized in the epidemiology of amebiasis. Occurrence of other protozoans and helminths was infrequent and rates were comparable in both groups.

127. **The Treatment of Human Helminthiases with Piperazine Citrate.** J. C. SWARTZWELDER, J. H. MILLER AND R. W. SAPPENFIELD, Louisiana State University School of Medicine, New Orleans.

*Enterobius vermicularis* was eliminated in 91 percent of a series of 33 patients treated for 14 consecutive days with piperazine citrate (Syrup of Antepar). Ninety-six percent of a series of 27 patients were treated successfully with the same anthelmintic for two alternate weeks (seven days' treatment, seven days without therapy, seven days' additional medication). The difference between the therapeutic results with these two regimes is not statistically significant. Complaints during therapy were infrequent and mild in both groups of patients. The total daily dosage was 30 to 35 mg. of the hexahydrate equivalent of piperazine citrate syrup per pound (divided in two equal doses). The maximum daily dose was 2.0 gm.

Twenty-two patients with ascariasis were treated for one day with piperazine citrate. The dosage employed was approximately 70 mg. per pound with a maximum of 3.0 gm. No fasting or purgation was employed. Stoll egg counts were performed on stools before and after treatment. Counts before therapy ranged from 1,300 to 53,800 *Ascaris lumbricoides* eggs per ml. The infection was eliminated from 18 of 22 patients. The egg counts of the remaining four patients were reduced from 50 to 90 percent. There were no untoward reactions to the drug when given in a single dose in the amount indicated above. Piperazine citrate syrup has been employed effectively in the treatment of cases of partial intestinal obstruction due to ascariasis.

Piperazine citrate failed to eliminate infections with *Strongyloides stercoralis, Trichuris trichiura* and *Hymenolepis nana.*

128. **The Early Migration and Growth of *Nippostrongylus muris* in the Rat.** DONALD W. TWOHY, The Johns Hopkins University.

Pepsin digestions of the skins and lungs of subcutaneously infected rats and aliquot counts of larvae-tissue suspensions were employed to determine the total *Nippostrongylus muris* larvae per tissue site.

The larvae were first noted in the lungs 11 hours after infection and by 15 hours the majority of the larvae had completed the migration—except for 30 to 40 percent of the recovered larvae still present in the skin at 18 to 19 hours. It is doubted that these latter larvae ever complete the migration. The first larvae were found in the digestive tract 41.5 hours after infection and by 59 hours over 90 percent of the larvae had completed the migration. Sometime between 45 and 50 hours 50 percent of the larvae reached the digestive tract. Both migrations followed a sigmoid curve when the larval recoveries for each migration site were plotted against time.

When larvae were allowed to penetrate the skin, recoveries varied greatly between experiments and penetration of many larvae was delayed.

Larvae in the skin first underwent a decrease in total length, followed by an increase toward the end of their inhabitation of the skin. The pattern of growth during the first few hours in the lung was confused by the appearance of additional migrants, but a rapid growth was apparent after 18 or 19 hours. This continued until about 32 hours when the larvae began the process of the third molt. Rapid growth was resumed by larvae reaching the digestive tract.

129. **Effects of Sodium Borate on Swine Nodular-Worm** (*Oesophagostomum dentatum*) **Infective Larvae in Soil.** JOSEPH E. ALICATA, University of Hawaii.

In an earlier paper (Alicata, 1953, Amer. Jour. Vet. Res. **14**: 563–570), it was reported that the sodium borate product "Polybor-3" (Pacific Coast Borax Co., Los Angeles) when sprayed on soil under simulated and shaded natural conditions at the rate of five pounds to 100 square feet, was effective in 20 days in destroying most of the kidney-worm (*Stephanurus dentatus*) larvae in soil up to a depth of one inch. Similar experiments which have been carried out with the use of infective nodular-worm larvae have revealed also that most of the larvae were killed in 20 days. These larvae, however, appeared to show more resistance to the action of this chemical than those of kidney-worms.

In the present study, three trials were conducted at different times. Each trial was run in triplicate simultaneously and with respective controls. Approximately 10,000 larvae were placed in each small wire basket that was set in soil as outlined in the paper cited above. The soil was lightly sprayed with water once a day.

Test results show that the number of viable larvae recovered from the soils in the nine wire baskets, 20 days after treatment, averaged 471 for the treated soils and 5,228 for the untreated soils. The number of viable larvae recovered from each of the nine treated soils compared with the number from corresponding untreated controls varied from 4.0 to 15.9 percent. These results, compared with those already reported for kidney-worm larvae show that the viable larvae recovered 20 days after treatment was 0.1 to 0.2 percent.

130. **Parasites of Amphibia.** A. C. WALTON, Knox College, Galesburg, Illinois.

The following records have been added to the Catalog of Amphibian Parasites since last year: —PROTOZOA: *Nyctotherus* sp? of Kifer, 1953, from *Bufo viridis*—Poland; *Trichomonas augusta*, from *Atelopus varius zeteki*—Panama; *Bufo b. asiaticus*—S.E. Asia; *B. terrestris*, *B. woodhousii*, *Hyla arenicolor*, and *H. squirella*—U.S.A.; *Microhyla olivacea*—S.E. Asia; *Rana virgatipes*, and *Triturus pyrrhogaster*—U.S.A. (the latter introduced). ACANTHOCEPHALA: *Acanthocephalus bufonis* from *Bufo asper* and *Rana tigerina*—Celebes; *Acanthocephala lucidus* from *Bufo b. japonicus*—Japan; and larval *Gordiorhynchus hylae* from *Hyla aurea* and *H. caerulea*—Australia. CESTODA: *Baerietta claviformis* from *Rana temporaria ornativentris*—Japan; *Baerietta montana* from *Bufo b. japonicus*—Japan; and spargana of *Diphyllobothrium erinacei* from *Hyla caerulea* and *H. latopalmata*—Australia. NEMATODA: *Cosmocerca japonica* from *Bufo b. japonicus* and *Rana temporaria ornativentris*—Japan; *Cosmocercoides dukae* from *Desmognathus f. fuscus*—U.S.A.; *Cosmocercoides pulcher* from *Rana temp. ornativentris*—Japan; the *Oxysomatium filiformis* of Grabda, 1953, from *Salamandra salamandra*—Europe; *Spironoura* sp? of Fischthal, 1955, from *Gyrinophilus p. porphyriticus*—U.S.A.; and *Rhabdias montana* from *Rana temp. ornativentris*—Japan. TREMATODA: larval *Echinoparyphium flexum* from *Hyla crucifer*, *Pseudacris nigrita triseriata*, *Rana clamitans*, *R. pipiens*, and *R. sylvatica*—U.S.A.; larval *Paralepoderma brumpti*, *P. cloacicola*, and *P. progeneticum* from *Triturus palmatus*—Europe; and larval *Prohemistomum expeditum* from *Rana esculenta ridibunda*—N. Africa.

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